SYMPOSIUM

The Proteomics of Sickle Cell Disease: Profiling of Erythrocyte Membrane Proteins by 2D-DIGE and Tandem Mass Spectrometry

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Quantitative changes in the red blood cell membrane proteome in sickle cell disease were analyzed using the two-dimensional fluorescence difference gel electrophoresis 2D-DIGE technique. From over 500 analyzed two-dimensional gel spots, we found 49 protein gel spots whose content in sickle cell membranes were changed by at least 2.5-fold as compared to control cells. In 38 cases we observed an increase and in 11 cases a decrease in content in the sickle cell membranes. The proteins of interest were identified by in-gel tryptic digestion followed by liquid chromatography in line with tandem mass spectrometry. From 38 analyzed gel spots, we identified 44 protein forms representing different modifications of 22 original protein sequences. The majority of the identified proteins fall into small groups of related proteins of the following five categories: actin accessory proteins—four proteins, components of lipid rafts—two proteins, scavengers of oxygen radicals—two proteins, protein repair participants—six proteins, and protein turnover components—three proteins. The number of proteins whose content in sickle RBC membrane is decreased is noticeably smaller, and most are either components of lipid rafts or actin accessory proteins. Elevated content of protein repair participants as well as oxygen radical scavengers may reflect the increased oxidative stress observed in sickle cells. Exp Biol Med 230:787–792, 2005

Key words: sickle cell disease; RBC membrane proteins; proteomics

Introduction

Sickle cell disease (SCD) is the first hereditary disease where the molecular basis of a genetic disorder was elucidated. A point mutation replacing thymine for adenine in the beta globin gene on chromosome 11 causes the single amino acid substitution replacing valine for glutamic acid in residue 6 of beta globin (1). As a result of this genetic mutation, normal hemoglobin, HbA, is replaced by sickle cell anemia hemoglobin, HbS. Despite the same genetic defect in all homozygous SS patients, there is significant diversity in clinical severity and outcome. That is, there is considerable phenotypic heterogeneity among individuals with identical alleles at the beta globin locus (2), and the correlation between genotype and phenotype is unclear (3). Clearly other epigenetic factors are responsible for the diversity in clinical severity. Vasoocclusion results from association of red blood cells with white blood cells and both attaching to the endothelial wall (aided by plasma components) of the blood vessel blocking blood flow (4). The result is the sickle cell vasoocclusive crisis, where there is an inverse correlation between crisis rate and life span of the SCD patient (5). Therefore, we believe that the best way to understand differences in clinical severity and outcome for sickle cell patients is to obtain a complete understanding of specific alterations in the proteome of red blood cells, white blood cells, plasma, and the vascular endothelium. As a prelude to this work, we have analyzed the normal erythrocyte proteome by liquid chromatography in line with tandem mass spectrometry (LC/MS/MS) (6).

In this study, we analyzed quantitative changes in the RBC membrane proteome caused by SCD. Protein profiling was performed by the two-dimensional fluorescence differ-

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ence gel electrophoresis (2D DIGE) technique in combination with LC/MS/MS.

Experimental Procedures

Sample Preparation. Human blood samples were collected from healthy (control) donors (AA) and homozygous (SS) sickle cell patients at the UT Southwestern Comprehensive Sickle Cell Center after receiving informed consent. Peripheral whole blood was collected in vacutainer tubes containing lithium heparin, sufficient for 10 ml of blood, and used within 24 hrs. The RBCs were sedimented at 1000 *g* for 10 mins at 4°C and resuspended in PBS (10 m*M* $NaPO_4$, pH 7.6, 150 m*M* NaCl) to the original volume (~10 ml) four times. Each time, the upper 1–2-mm layer of packed cells was aspirated along with liquid phase to remove white blood cells. RBCs were transferred to 50 ml centrifugal tubes (2–3 ml packed cells per tube) and washed with PBS at 4°C three times: the cells were resuspended in 10 volumes of PBS and sedimented at 2000 *g* for 10 mins. Cell membranes were prepared from washed RBCs as described (7). The membranes containing ~4 mg of protein/ml were freeze-dried and solubilized in lysis buffer containing 30 m*M* Tris-HCl, pH 8.5, 7 *M* urea, 2 *M* thiourea, and 2% (w/v) nonionic detergent ASB 14 at 3–5 mg of protein/ml. Protein concentration was determined with Protein Assay Reagent (Bio-Rad, Hercules, CA).

Protein Labeling. Membrane proteins (0.08 mg) solubilized in lysis buffer were minimally labeled with Cy3 or Cy5 fluorophores (Amersham Biosciences, Piscataway, NJ) according to the manufacturer's protocol. The control AA and SS proteins were derived from the blood samples collected from a single healthy donor (adult male) and sickle cell patient (18-year-old male), respectively. Equal amounts of SS and control AA proteins (0.08 mg each) labeled with the two different fluorophores (Cy5 and Cy3, respectively) were mixed before separation on a 2D gel in a sample/control (or SS/AA) experiment. In a parallel control/control (or AA/AA) experiment, equal amounts of the same control AA proteins (0.08 mg each) labeled with two different fluorophores were mixed and analyzed. To confirm the differences in SS/AA protein contents observed in the first experiment, the second sample/control experiment was carried out using the same control AA and SS proteins and similar labeling.

2D Gel Electrophoresis. Mixtures of labeled proteins (total 0.16 mg) were separated by 2D gel electrophoresis as described (8). The first dimension, isoelectric focusing (IEF), was performed in a 13-cm Immobiline DryStrip with a nonlinear pH 3–10 gradient using Ettan IPGphor II (Amersham Biosciences) at 20°C. Immobiline strip rehydration was performed for 12 hrs in a rehydration buffer (1% Pharmalyte 3–10 NL, 7 *M* urea, 2 *M* thiourea, 2% ASB 14, and 2 mg/ml dithiothreitol-DTT) containing 0.16 mg mix of Cy3- and Cy5-labeled proteins. Isoelectric focusing was performed in three steps: at 500 V for 1 hr, at 1000 V for 1 hr, and at 8000 V for 33,300 V·hr. Prior to second dimension, Immobiline strip with separated proteins was equilibrated and reduced in a solution containing 50 m*M* Tris-HCl, pH 8.6 buffer, 2% SDS, 30% glycerol, and 5 mg/ml DTT at 90°C for 1 min followed by an equilibration and protein alkylation (carbamidomethylation) at room temperature in a solution with the same Tris-HCl buffer containing 6 *M* urea, 2% SDS, 30% glycerol, and 20 mg/ml iodoacetamide for 10 min. After equilibration and alkylation, the proteins separated by IEF were further separated by SDS-PAGE on a 10% polyacrylamide gel. The separation was performed in a Hoefer SE 600 unit (Amersham Biosciences) at 25 mA/gel constant current until the dye front migrated out of the gel. Where indicated, the gels were stained with Sypro Ruby (Molecular Probes) according to the manufacturer's protocol.

Gel Image Analysis. The separated proteins labeled with Cy3 and Cy5 fluorophores were detected in gels using a 2920 2D-Master Imager (Amersham Biosciences). After detection, the identical Cy3- and Cy5-labeled proteins migrating to the same 2D spot were quantified based on the corresponding fluorescence intensities, and their molar ratios were calculated using DeCyder Differential In-Gel Analysis software (Amersham Biosciences).

Protein Identification. Selected 2D gel spots were analyzed for protein identification. Proteins were identified through identification of corresponding tryptic peptides. The selected spots were excised from Sypro Ruby–stained 2D gels using Ettan Spot Picker (Amersham Biosciences). Proteins in the excised gel pieces were digested using in-gel trypsin digestion kit (Pierce, Rockford, IL), and corresponding tryptic digests (~0.025 ml) were collected according to the manufacturer's protocol. Peptides in each tryptic digest were separated and identified by LC/MS/MS and database search.

Mass Spectrometry. The LC/MS/MS analysis was performed using a Surveyor high-performance liquid chromatography (HPLC) system connected through PepFinder kit (with peptide trap and 99:1 flow splitter) to a LCQ DECA XP ion trap mass spectrometer with a nanospray ionization source (ThermoFinnigan, San Jose, CA). Peptides in the tryptic digest (5 μl) were separated by reverse-phase HPLC on a PicoFrit BioBasic C18 column (New Objectives, 0.075 × 100 mm) at 0.7 μl/min flow rate. Water and acetonitrile with 0.1% formic acid each were used as solvents A and B, respectively. The gradient was started and kept for 10 min at 0% B, then ramped to 60% B in 60 min, and finally ramped to 90% B for another 15 min. The eluted peptides were analyzed in data-dependent MS experiments ("big three") with dynamic exclusion (6). The spray voltage was set at 1.6 kV; the ion transfer capillary temperature was set at 180°C.

Database Search. Each acquired MS/MS spectrum was searched against the NCBI nonredundant protein sequence database (nr.fasta, May 2004), using the SEQUEST software tool (9, 10). Database search parameters and peptide identification criteria used were as described (6). The proteins were identified through at least three identified tryptic peptides.

Results and Discussion

Changes in the RBC membrane proteome in SCD were analyzed using the 2D-DIGE technique as described in *Materials and Methods*. Equal amounts of total RBC membrane protein derived from control AA and SS sickle

cells were minimally labeled with two different fluorescent dyes, mixed, and separated on a 2D gel by isoelectric focusing followed by SDS-PAGE. The identical AA and SS proteins migrating to the same 2D spots were detected and quantified based on their fluorescence intensities, and corresponding SS/AA protein ratios were determined. An SS/AA ratio shows the relative content of a particular protein in the SS sickle RBC membrane as compared to the AA control, and its value indicates the difference in protein abundance (increase or decrease) caused by SCD. To determine the variability inherent in the method, we performed a parallel AA/AA (control/control) experiment. In that experiment, equal amounts of the same control AA proteins were labeled with two different fluorescent dyes, mixed, and analyzed. We wanted to determine the variance from the predicted AA/AA ratio of 1. The variation in the determined AA/AA ratios and frequency distribution showed that 99.8% of all determined ratios were within 2.5-fold difference from the expected value of 1. Based on this analysis, we required greater than a 2.5-fold difference in SS/AA protein ratio to consider the change as statistically significant.

Frequency distribution of SS/AA ratios determined in different spots of 2D gel is presented in Figure 1. From over 500 analyzed gel spots, we selected those where the SS protein was changed by at least 2.5-fold as compared to the AA counterpart. The changes, found in 49 of the selected gel spots, were confirmed in the second sample/control experiment, carried out with the same control AA and SS proteins. In 38 cases we observed an increase in the SS sickle cell membrane and in 11 cases a decrease. These 49 gel spots were selected for further analysis for protein identification. Using the Ettan Spot Picker, we were able to localize 40 (out of 49) selected protein spots on the Sypro Ruby–stained 2D gel, excise them, and analyze them for protein identification. No protein was identified in two of the 40 analyzed gel spots. The 2D gel and 38 spots where proteins were identified are shown in Figure 2.

The analyzed gel spots, determined SS/AA ratios, molecular weights (Mr), and isoelectric points (pI) as well as proteins identities are listed in Table 1. The names of identified proteins, corresponding gi numbers, and Mr and pI derived from the protein sequences are given from the SEQUEST search summary. The determined apparent molecular masses of identified proteins ranged from 22 to 92 kDa; the apparent pI varied from 4.46 to 8.72, though pI derived from identified protein sequences varied from 4.25 to 10.07.

Eight of the identified proteins were found in more than one spot corresponding to similar molecular masses with different pI: ankyrin (gi 105337 or 10947038) was found in

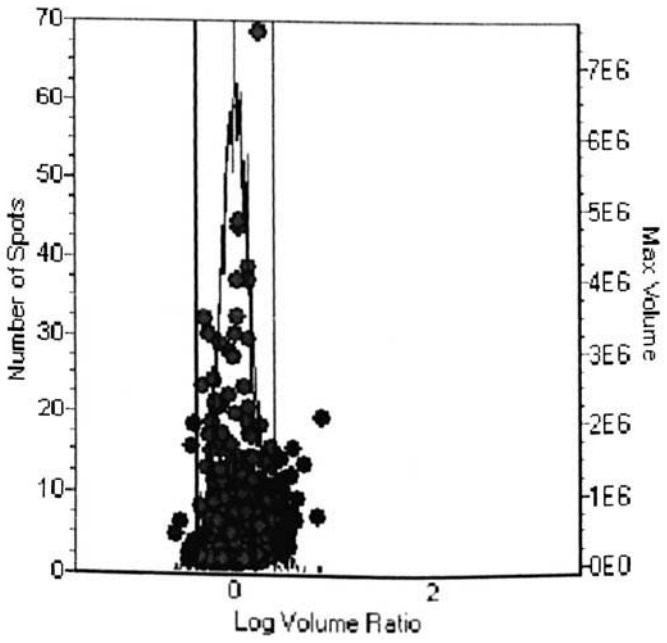

Figure 1. Frequency distribution of SS/AA protein ratios. A mixture of equal amounts of AA and SS RBC membrane proteins (0.08 mg each), minimally labeled with Cy3 and Cy5 fluorescent dyes, respectively, was separated on a 2D gel by isoelectric focusing (IEF) followed by SDS-PAGE. The identical AA and SS proteins migrating to the same 2D gel spots were quantified, and SS/AA protein ratios were determined. Each analyzed 2D gel spot is presented as a circle: its position corresponds to the logarithm of SS/AA protein ratio and the abundance of the dominant (AA or SS) protein; the volume scale on the right Y-axis is equivalent to protein abundance. The spots where abundance of SS protein was decreased or increased as compared to the AA counterpart by at least 2.5-fold are presented in red or blue, respectively. The rest of the analyzed gel spots are presented in green. The blue bell-shaped line shows the frequency distribution of logarithms of determined SS/AA protein ratios.

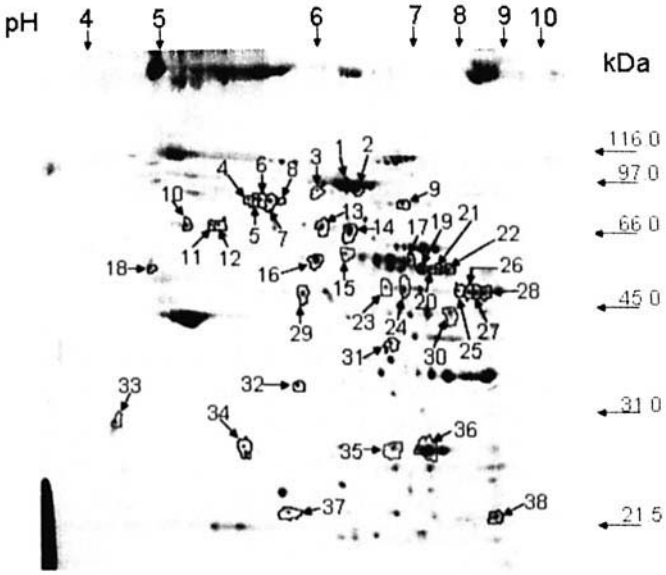

Figure 2. Separation of RBC membrane proteins on a 2D gel. A mixture of equal amounts of AA and SS RBC membrane proteins (0.08 mg each), minimally labeled with Cy3 and Cy5 fluorescent dyes, respectively, was separated on a 2D gel by isoelectric focusing (IEF) followed by SDS-PAGE. The IEF was performed in a 13-cm Immobiline DryStrip with a nonlinear pH 3–10 gradient. The proteins were further separated by SDS-PAGE on a 10% polyacrylamide gel. The identical AA and SS proteins migrating to the same 2D spots were detected and quantified based on corresponding fluorescence intensities, and SS/AA protein ratios were determined. The Cy3 image of the gel is presented in the figure. The gel spots selected for protein identification are indicated. The red and blue borders indicate the spots where abundance of SS protein was decreased or increased, respectively, as compared to the AA counterpart, by at least 2.5-fold.

Table 1. Proteins Changing in SS RBC Membranes

Spot no.	Identified protein	gi no.	SS/AA ratio[a]	Mr/pI calculated	Mr/pI experimental
1	Ankyrin 1, erythrocyte splice form 2	105337	+4.9	207,265/6.00	92,200/6.15
	or Ankyrin 1 isoform 1; ankyrin R	10947038	+4.9	203,475/6.00	92,200/6.15
2	Ankyrin 1, erythrocyte splice form 2	105337	+3.9	207,265/6.00	92,200/6.22
	or Ankyrin 1 isoform 1; ankyrin R	10947038	+3.9	203,475/6.00	92,200/6.22
3	Protein 4.1 (band 4.1)	14916944	−3.2	97,587/4.25	89,200/5.98
4	Protein 4.1 (band 4.1)	14916944	+3.3	97,587/4.25	82,700/5.60
5	Protein 4.1 (band 4.1)	14916944	+3.6	97,587/4.25	82,700/5.63
6	Protein 4.1 (band 4.1)	14916944	+3.5	97,587/4.25	82,700/5.67
7	Protein 4.1 (band 4.1)	14916944	+6.6	97,587/4.25	82,700/5.72
8	Protein 4.1 (band 4.1)	14916944	+3.8	97,587/4.25	82,700/5.78
9	Protein 4.1 (band 4.1)	14916944	+2.7	97,587/4.25	80,000/6.70
10	Heat shock 70-kDa protein 8 isoform 1	5729877	+7.3	71,126/4.25	68,200/5.23
11	Heat shock 70-kDa protein 1	462325	+2.8	70,338/4.25	68,200/5.38
12	Heat shock 70-kDa protein 1	462325	+3.5	70,338/4.25	68,200/5.43
13	Chaperonin containing TCP1, subunit 2	5453603	+3.4	57,831/6.00	67,100/6,02
14	Chaperonin containing TCP1, subunit 6A (zeta 1)	4502643	+2.8	58,481/6.00	66,000/6.16
15	Chaperonin containing TCP1, subunit 6A (zeta 1)	4502643	−2.8	58,481/6.00	58,100/6.14
	and EH-domain containing 1; testilin	30240932		60,684/6.00	58,100/6.14
16	Chaperonin containing TCP1, subunit 2	5453603	+2.9	57,831/6.00	56,500/5.97
17	Catalase	4557014	+3.1	59,984/6.00	56,500/6.80
18	Tubulin alpha 6	14389309	+2.6	50,580/4.25	54,300/4.84
19	Chaperonin containing TCP1, subunit 7 (eta)	5453607	+3.5	59,880/8.33	54,300/7.06
	and T-complex protein 1, delta	1729870		58,353/8.33	54,300/7.06
20	Chaperonin containing TCP1, subunit 7 (eta)	5453607	+3.4	59,880/8.33	54,300/7.28
	and T-complex protein 1, delta	1729870		58,353/8.33	54,300/7.28
21	Chaperonin containing TCP1, subunit 7 (eta)	5453607	+2.9	59,880/8.33	54,300/7.40
	and T-complex protein 1, delta	1729870		58,353/8.33	54,300/7.40
22	Chaperonin containing TCP1, subunit 7 (eta)	5453607	+4.2	59,880/8.33	54,300/7.66
23	Flotilin 1	5031699	−3.3	47,583/7.17	48.900/6.43
24	Flotilin 1	5031699	−2.8	47,583/7.17	48.900/6.72
25	ATP-synthase, alpha subunit	4757810	+2.6	59,865/10.07	48.900/7.88
26	ATP-synthase, alpha subunit	4757810	+4.1	59,865/10.07	48.900/8.10
27	ATP-synthase, alpha subunit	4757810	+5.1	59,865/10.07	48.900/8.29
28	ATP-synthase, alpha subunit	4757810	+4.0	59,865/10.07	48.900/8.48
29	Chaperonin containing TCP1, subunit 2	5453603	+3.4	57,831/6.00	47,800/5.90
30	Dematin (band 4.9)	22654240	−2.6	45,629/10.07	44,100/7.71
31	Proteasome 26S ATPase subunit 6	24430160	+2.6	44,456/7.17	39,300/6.51
32	Proteasome alpha 1 subunit, isoform 1	23110935	+2.5	30,525/6.00	33,900/5.86
33	Tropomyosin 3	24119203	−2.6	29,261/4.25	29,600/4.46
34	Stomatin isoform a (band 7.2)	38016911	+2.7	31,902/8.33	27,400/5.56
35	Stomatin isoform a (band 7.2)	38016911	−3.8	31,902/8.33	27,400/6.54
36	Stomatin isoform a (band 7.2)	38016911	−3.1	31,902/8.33	27,400/7.16
37	Peroxiredoxin 3 isoform b	32483377	+2.9	25,838/7.04	22,200/5.81
38	Peroxiredoxin 1	4505591	+2.8	22,339/8.33	22,000/8.72
	and RAB-8b protein	7706563		23,755/10.07	22,000/8.72
	and Proteasome beta 1 subunit	4506193		26,718/8.33	22,000/8.72

[a] SS/AA ratio of "*n*" with "+" or "−" sign, respectively, indicates *n*-fold increase or decrease of SS protein as compared to AA counterpart.

spots 1 and 2; protein 4.1 (gi 14916944) in spots 4–8; heat shock 70-kDa protein 1 (gi 462325) in spots 11 and 12; chaperonin containing TCP1, subunit 7 (eta) (gi 5453607), in spots 19–22; T-complex protein 1, delta (1729870), in spots 19–21; flotilin 1 (5031699) in spots 23 and 24; ATP-synthase (gi 4757810) in spots 25–28; and stomatin isoform a (band 7.2, gi 38016911) in spots 34–36. This most probably reflects post-translational modifications altering protein pI with little effect on its molecular mass (e.g., phosphorylation). The nature of the modifications has not yet been established.

Three of the identified proteins were found in more than one gel spot corresponding to different molecular masses: protein 4.1 (gi 14916944) was found in spots 3, 4–8, and 9; chaperonin containing TCP1, subunit 2 (gi 5453603), in spots 13, 16, and 29; and chaperonin containing TCP1, subunit 6A (zeta) (gi 4502643), in spots 14 and 15. In general, this may reflect post-translational modifications in a protein altering its molecular mass (e.g., glycosylation), alternative splice forms, or partial degradation of a protein. For the proteins mentioned, alternative transcriptional splice variants of the gene have

been observed (11–13). Ankyrin (molecular mass ~200 kDa) was found in gel spots 1 and 2 corresponding to a molecular mass of 92.2 kDa. These spots most probably represent partial degradation products of ankyrin. We have previously described a degradation product of ankyrin at this molecular mass (14, 15). More than one protein was found in gel spots 15, 19, 20, 21, and 38. In such cases, the determined SS/AA ratio may reflect (i) the similar difference in SS/AA contents for all found proteins or (ii) the minimal difference in SS/AA contents for at least one protein, though we cannot specify which one(s). In the 38 analyzed gel spots, we identified 44 proteins representing 22 original protein sequences. It should be noted that a 2D gel spot may represent one of several possible modifications to a specific protein. Therefore, a significant change found in the quantity of such a protein spot may be the result of a quantitative redistribution of the protein's modifications or protein content. For the red blood cell, which lacks the ability to synthesize protein, a change in content would require a difference in protein synthesis or degradation during erythropoiesis.

The majority of proteins that change at least 2.5-fold fall into small groups of related proteins in the following five categories: actin accessory proteins—four proteins, components of lipid rafts—two proteins, scavengers of oxygen radicals—two proteins, protein repair participants—six proteins, and protein turnover components—three proteins. The 22 identified original proteins and protein categories are listed in Table 2. It should be noted that chaperonin containing TCP1, subunit 7 (eta) (gi 5453607), and T-complex protein 1, delta (gi 1729870), were found in the same 2D gel spot with SS/AA ratios of higher than 2.5. We included both proteins in one of the protein groups listed in Table 2, though we cannot exclude the possibility that the determined difference in the SS/AA content is a characteristic of only one of the two proteins. The same is true for other gel spots that also contain multiple proteins, as indicated in Table 2. Of the four proteins of actin accessory group, tropomyosin and dematin are essential components of the RBC membrane skeleton attached to the membrane via ankyrin and protein 4.1 (16). The protein repair participants group includes members of the heat shock protein 70-kDa family and the chaperonin containing TCP1 complex. The complex also known as the TCP1 ring complex (TRiC) consists of two identical stacked rings, each containing eight different proteins (13). Unfolded polypeptides including actin and tubulin enter the central cavity of the complex and are folded in an ATP-dependent manner (13). The heat shock proteins mediate the folding of newly translated polypeptides and in cooperation with other chaperones stabilize preexistent proteins against aggregation (17). Flotilin 1 and stomatin (band 7.2) are major proteins of lipid rafts, detergent-resistant, cholesterol- and sphingolipid-rich membrane domains that are involved in important cellular processes such as signal transduction and intracellular trafficking (18, 19). Stomatin is also a regulator of monovalent cation flux across the membrane (20). Oxygen radical scavengers, peroxiredoxins are a family of antioxidant enzymes controlling hydrogen peroxide content and play a protective role in cells (21). All observed protein turnover

Table 2. The Altered Proteins in SS RBC Membranes Placed in Functional Categories

No.	Identified protein	gi no.	Spot no.[a] Down	Spot no.[a] Up	Protein groups
	Ankyrin 1, erythrocyte splice form 2	105337		1, 2	Actin accessory proteins
1	or Ankyrin 1 isoform 1; ankyrin R	10947038			
2	Protein 4.1 (band 4.1)	14916944	3	4–9	
3	Dematin (band 4.9)	22654240	30		
4	Tropomyosin 3	24119203	33		
5	Heat shock 70-kDa protein 8 isoform 1	5729877		10	Protein repair participants
6	Heat shock 70-kDa protein 1	462325		11, 12	
7	Chaperonin containing TCP1, subunit 2	5453603		13, 16, 29	
8	Chaperonin containing TCP1, subunit 6A (zeta 1)	4502643	15[b]	14	
9	Chaperonin containing TCP1, subunit 7 (eta)	5453607		19–22[c]	
10	T-complex protein 1, delta	1729870		19–21[c]	
11	Flotilin 1	5031699	23, 24		Lipid rafts
12	Stomatin isoform a (band 7.2)	38016911	35, 36	34	
13	Proteasome 26S ATPase subunit 6	24430160		31	Protein turnover components
14	Proteasome alpha 1 subunit isoform 1	23110935		32	
15	Proteasome beta 1 subunit	4506193		38[d]	
16	Peroxiredoxin 3 isoform b	32483377		37	Scavengers of oxygen radicals
17	Peroxiredoxin 1	4505591		38[d]	
18	EH-domain containing 1; testilin	30240932	15[b]		Others
19	Catalase	4557014		17	
20	Tubulin alpha 6	14389309		18	
21	ATP-synthase, alpha subunit	4757810		25–28	
22	RAB-8b protein	7706563		38[d]	

[a] 2D gel spot number where indicated SS protein was decreased (Down) or increased (Up) as compared to AA counterpart by at least 2.5-fold.
[b,c,d] The identical superscripts indicate proteins found in the same gel spot.

components are proteasome subunits. The proteasome is a multicatalytic proteinase complex distributed throughout eukaryotic cells and cleaves peptides in an ATP/ubiquitin-dependent process (22). Though absence of proteasomes in RBCs was reported (23), some of its subunits were recently found in RBCs (6, 24). The number of protein spots whose content was decreased in sickle RBC membrane was smaller and most of them are either components of lipid rafts or actin accessory proteins. Elevated contents of protein repair participants as well as oxygen radical scavengers may reflect the increased demand for the proteins under the harsh oxidative conditions in sickle cells. Sickle cells contain two to three times as many oxygen radicals as control red blood cells (25) with greatly diminished reduced glutathione (GSH) (26). Indeed, there is an inverse correlation between sickle cell density and reduced glutathione levels, with the highest density SS RBCs containing no measurable GSH (26). Therefore, the sickle cell is in double jeopardy for oxidative damage to protein and lipid components (27). From the current protein profiling experiments, it appears that the adaptive response of sickle cells is to increase the levels of proteins that scavenge oxygen radicals, proteins that help refold damaged protein, and proteasomal proteins involved in the turnover of oxidatively damaged proteins. The increased levels of proteins involved in damage control is due to either protein modifications that can occur in the mature RBC or increases in protein levels that would have occurred earlier in erythropoiesis.

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