Time-course investigation of SAGM-stored leukocyte-filtered red bood cell concentrates: from metabolism to proteomics

Angelo D'Alessandro,[1] Gian Maria D'Amici,[1] Stefania Vaglio,[2] and Lello Zolla[1]

[1]Department of Environmental Sciences, University of Tuscia, Largo dell'Università, and [2]Italian National Blood Centre, Italian National Institute of Health, Rome, Italy

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Online Supplementary Design and Methods

Sample collection

Whole blood (450 mL±10%) was collected from healthy volunteer donors into citrate-phosphate-dextrose (CPD) anticoagulant (63 mL) and leukodepleted. After separation of plasma by centrifugation, red blood cells (RBC) were suspended in 100 mL of saline, adenine, glucose, mannitol (SAGM) additive solution. We studied RBC units collected from eight donors [male = 4, female = 4, age 45±11.5 (mean±SD)] who signed informed consent according to the declaration of Helsinki. RBC units were stored under standard blood bank conditions (1-6 °C) and samples were removed aseptically for analysis every week from day 0 until day 42 of storage.

Biochemical analyses

Determination of intracellular pH

Red cell pellets obtained by centrifuging 600 μL of suspension in a nylon tube at 30,000×g for 10 min were frozen, thawed over 5 min and then refrozen. To prevent an acid shift observed when samples are kept unfrozen, triplicate measurements of pH were made immediately after a second thawing of each lysate with a Radiometer pH glass capillary electrode maintained at 20°C and linked to a Radiometer PHM acid-base analyzer.

Metabolomics

Samples from the eight units were extracted following the protocol by Sana *et al.*[1] with minor modifications.[2]

Rapid resolution reversed-phase high performance liquid chromatography

An Ultimate 3000 Rapid Resolution HPLC system (LC Packings, DIONEX, Sunnyvale, USA) was used to separate metabolites. The system has a binary pump and vacuum degasser, well-plate autosampler with a six-port micro-switching valve, and a thermostated column compartment. A Dionex Acclaim RSLC 120 C18 column 2.1 mm×150 mm, 2.2 μm was used to separate the extracted metabolites. Acetonitrile, formic acid, and HPLC-grade water, purchased from Sigma Aldrich (Milan, Italy). The liquid chromatography (LC) parameters were as follows: injection volume, 20 μL; column temperature, 30°C; and flow rate of 0.2 mL/min. The LC solvent gradient and timetable were identical during the whole period of the analyses. A 0–95% linear gradient of solvent A (0.1% formic acid in water) to B (0.1% formic acid in acetonitrile) was employed over 15 min followed by a solvent B hold of 2 min, returning to 100% A in 2 min and a 6-min post-time solvent A hold.

Electrospray ionization mass spectrometry

Metabolites were directly eluted into a High Capacity ion Trap HCTplus (Bruker-Daltonik, Bremen, Germany). Mass spectra for metabolite extracted samples were acquired in positive and negative ion modes, as previously described.[2] The electrospray ionization (ESI) capillary voltage was set at 3000V in (+) ion mode. The liquid nebulizer was set to 30 psig and the nitrogen drying gas was set to a flow rate of 9 L/min. Dry gas temperature was maintained at 300°C. Internal reference ions were used to maintain mass accuracy continuously. Data were acquired at a rate of 5 spectra/s with a stored mass range of m/z 50–1500. Data were collected using Bruker Esquire Control (v. 5.3 – build 11) data acquisition software. In multiple reaction monitoring (MRM) analysis, the m/z of interest were isolated, fragmented and monitored (either the parental and fragment ions) throughout the whole RT range. Validation of HPLC online mass spectroscopy (MS)-eluted metabolites was performed by comparing transition fingerprints, upon fragmentation and matching against the standard metabolites through direct infusion with a syringe pump (infusion rate 4 μL/min). Standard curve calibration was performed on precursor and fragment ion signals. Only the former were adopted for quantification, as precursor ion signals guaranteed higher intensity and thus improved limit of detection (LOD) and quantification of metabolites of interest.[2] However, transitions were monitored in independent runs to validate each detected metabolite.

Metabolite analysis and data elaboration

Quantitative analyses of standard compounds were performed on MRM data against comparison to standard metabolite runs. Each standard compound was weighted and dissolved in nanopure water (18 mΩ). Calibration curves were calculated as previously reported.[1] In brief, each standard metabolite was run in triplicate, at incremental dilutions until the LOD was reached. The LOD for each compound was calculated as the minimum amount injected which gave a detector signal response higher than three times the noise (S/N > 3).

Standards (equal or greater than 98% chemical purity) D-

fructose and D-glucose 6-phosphate (G6P/F6P), D-fructose 1,6 biphosphate (FDP), glyceraldehyde phosphate (G3P), 1,3 and 2,3 diphosphoglycerate (DPG), phosphoenolpyruvic acid (PEP), L-lactic acid (LA), NADPH, phosphogluconolactic acid (PGL), ATP, NADH, glutathione (GSH), oxidized glutathione (GSSG), were purchased from Sigma Aldrich (Milan).

Standards were stored either at -25°C, 4°C or room temperature, following the manufacturers' instructions.

LC/MS data files were processed by Bruker DataAnalysis 4.0 (build 234) software. Files from each run were either analyzed as .d files or exported as mzXML files, to be further processed for spectra alignment, peak picking and quantification with InSilicos Viewer 1.5.4 (Insilicos LLC; Seattle, USA).

Data were further refined (normalization of treated/controls) and plotted with GraphPad Prism 5.0 (GraphPad Software Inc.). Results were plotted as fold-change variation values upon normalization to day 0 controls, as described by Nishino *et al.*[3]

Structural analyses

Hemolysis and osmotic fragility

Hemolysis was calculated following the method by Harboe.[4] Samples were diluted in distilled water and incubated at room temperature for 30 min to lyse red blood cells. Samples from lysed RBC were diluted 1/300 while supernatants were diluted 1/10 in distilled water. After stabilization for 30 min and vortex mixing (Titramax 100, Heidolph Elektro, Kelheim, Germany), the absorbance of the hemoglobin was measured at 380, 415 and 450 nm (PowerWave 200 Spectrophotometer, Bio-Tek Instruments, Winooski, Vermont, USA). The mean blank was subtracted and the corrected OD (OD) was calculated as follows: $2 \times OD_{415} - OD_{380} - OD_{450}$.

The RBC hemolysis curve was determined by osmotic fragility behavior using different NaCl solutions. A sample of 25 μL of blood was added to a series of 2.5 mL saline solutions (0.0 to 0.9% of NaCl). After gentle mixing and resting for 15 min at room temperature the RBC suspensions were centrifuged at 1500 rpm for 5 min. The absorbance of released hemoglobin into the supernatant was measured at 540 nm according to Kraus *et al.*[5]

Scanning electron microscopy

Scanning electron microscopic studies of RBC were performed using a JEOL JSM 5200 electron microscope. Blood samples were fixed in phosphate-buffered saline (pH 7.2–7.4) 2.5% glutaraldehyde for 1 h, washed twice in 0.1 M phosphate buffer (pH 7.2–7.4), and mounted on poly-L-lysine-coated glass slides. The glass slides were kept in a moist atmosphere for 1 h, washed in phosphate buffer, post-fixed in 1% osmium tetroxide for 1 h, rinsed in distilled water, and dehydrated in a graded ethanol series (50-70-90-100%). After critical-point drying with liquid CO_2 in a vacuum apparatus and covering with a gold-palladiumlayer, the samples underwent scanning electron microscopic analysis. The different cell shapes were identified using Bessis' classification.[6] The percentages of discocytes, echinocytes, sphero-echinocytes, stomatocytes, sphero-stomatocytes, and spherocytes were evaluated by counting 1000 to 1500 cells in randomly chosen fields. Reversible and irreversible shapes were determined according to Berezina *et al.*[7] RBC manifesting echinocyte and stomatocyte shapes are capable of returning to the discocyte shape under certain conditions. Thus, these RBC shape changes are considered potentially reversible transformations. In contrast, RBC assuming sphero-echinocyte, sphero-stomatocyte, spherocyte, ovalocyte, and degenerated shapes are irreversibly changed cells.

Proteomics

Red blood cell protein extraction

Human RBC membrane and cytosol proteins were extracted on day 0, day 14 and day 35 using a conventional method described by Olivieri *et al.*[8] with some modifications.[9] The RBC were isolated by centrifuging twice at 1000×g for 10 min. Packed cells were washed three times in 5 mM phosphate buffer pH 8.0, containing 0.9% w/v NaCl; they were then centrifuged at 300×g for 10 min, at 4 °C. RBC were split into two groups, one treated with the protocol described by D'Amici *et al.*,[10] and the other undergoing resuspension in 1 mL phosphate-buffered saline containing 100 mM N-ethylmaleimide (NEM), to avoid possible oxidation artifacts during cell preparation.[11] After 15 min of incubation at room temperature, cells were pelleted and then lysed with 9 volumes of cold 5 mM phosphate buffer pH 8.0 containing 1 mM EDTA, 1 mM phenylmethanesulfonyl fluoride (PMSF) and, in one of the two groups of samples, also 100 mM NEM. Cytosol was collected after centrifugation at 17,000×g for 20 min at 4°C and its protein content was estimated by the DC protein assay method (Bio-Rad, Hercules, CA, USA). Membranes were washed with the same buffer until free of hemoglobin and then, in order to remove non-specifically membrane-bound cytosolic proteins, were washed three times with 0.9% w/v NaCl and centrifuged at 17,000×g, for 20 min at 4 °C. Protein content was estimated by the bicinchoninic acid method[12] and ghosts prepared in this way were used for the following steps.

Two-dimensional electrophoresis

To remove lipids, proteins were precipitated from a desired volume of each sample with a cold mix of tri-n-butyl phosphate/acetone/methanol (1:12:1). After incubation at 4 °C for 90 min, the precipitate was pelleted by centrifugation at 2800g for 20 min at 4 °C. After washing with the same solution, the pellet was air-dried and then solubilized in the focusing solution containing 7 M urea, 2 M thiourea, 2% (w/v) ASB 14, 0.8% (w/v) pH 3-10 carrier ampholyte, 40 mM Tris, 5 mM TBP, 10 mM acrylamide, 0.1 mM EDTA (pH 8.5), 2% (v/v) protease inhibitor cocktail (Sigma-Aldrich), and 2 mM PMSF. Before focusing, the sample was incubated in this solution for 3 h at room temperature, under strong agitation. To prevent over-alkylation, acrylamide was destroyed by adding an equimolar amount of DTE. A total of 250 μL of the resulting protein solution was then used to rehydrate 13 cm long IPG 3-10 NL (Amersham Biosciences) for 8 h. IEF was carried out on a Multiphor II (Amersham Biosciences) with a maximum current setting of 50 μA/strip at 20 °C. The total product time voltage applied was 40 000 Vh for each strip. For the second dimension, the IPG strips were equilibrated for 30 min in a solution containing 6 M urea, 2% (w/v) sodium dodecylsulfate (SDS), 20% (v/v) glycerol, and 375 mM Tris-HCl (pH 8.8), with gentle agi-

tation. The IPG strips were then laid on a 5-16% T gradient SDS-polyacrylamide gel with 0.5% (w/v) agarose in the cathode buffer (192 mM glycine, 0.1% w/v SDS and Tris to pH 8.3). The anode buffer was 375 mM Tris-HCl, pH 8.8. The electrophoretic run was performed at a constant current (10 mA for 60 min, followed by 40 mA until the run was completed). During the whole run, the temperature was set at 13 °C. Proteins were visualized by staining with Coomassie Brilliant Blue G-250 stain.[12]

Image statistical analysis

Ninety-six stained gels (3 technical replicates × 8 biological replicates × 2 groups x 2 periods – day 0 and 35 – with or without NEM) were digitalized using an ImageScanner and LabScan software 3.01 (Bio-Rad Hercules, CA, USA). The two-dimensional electrophoresis (2-DE) image analysis was carried out and spots were detected and quantified using the Progenesis SameSpots software v.2.0.2733.19819 software package (Nonlinear Dynamics, Newcastle, UK). Each gel was analyzed for spot detection and background subtraction. Within-group comparisons of protein spot numbers were determined by repeated measures analysis. Among-group comparisons were determined by an analysis of variance (ANOVA) procedure in order to classify sets of proteins that showed a statistically significant difference with a confidence level of 0.05. Spots which were significantly different between groups (days 0 and 35 with NEM; days 0 and 35 without NEM) and not significantly different in the three technical replicate and eight biological replicate samples were identified as described below. All statistical analyses were performed with the Progenesis SameSpots software v.2.0.2733.19819 software package. After the background subtraction, spot detection and match, one standard gel was obtained for each group, at either at day 0 or 35, with or without NEM. These standard gels were then matched to yield information about the spots of differentially expressed proteins. Differential protein expression was considered statistically significant when the *P* value was less than 0.05 and the change in the photodensity of protein spots between day 0 and 35 samples (with or without NEM, independently) had to be more than 2-fold. Moreover, as protein fragments were the main changes to be expected, as previously reported,[13] we took into account only protein spots with a molecular weight below approximately 60 kDa.

In-gel digestion and protein identification by MS/MS

Protein spots were carefully excised from stained gels and subjected to in-gel trypsin digestion according to Shevchenko *et al.*[14] Peptide mixtures were separated using a nanoflow-HPLC system (Ultimate; Switchos; Famos; LC Packings, Amsterdam, The Netherlands). A sample volume of 10 μL was loaded by the autosampler onto a homemade 2 cm fused silica precolumn (75 μm I.D.; 375 μm O.D.; Reprosil C18-AQ, 3 μm, Dr. Maisch GmbH, Ammerbuch-Entringen, Germany) at a flow rate of 2 μL/min. Peptides were sequentially eluted using a flow rate of 200 nL/min and a linear gradient from Solution A (2% acetonitrile; 0.1% formic acid) to 50% of Solution B (98% acetonitrile; 0.1% formic acid) in 40 min over the precolumn in-line with a homemade 10-15 cm resolving column (75 μm ID; 375 μm OD; Reprosil C18-AQ, 3 μm, Dr. Maisch GmbH, Ammerbuch-Entringen, Germany). Peptides were eluted directly into a High Capacity Ion Trap (model HCTplus, Bruker-Daltonik, Germany). The general mass spectrometric parameters were as follows: capillary voltage, 1.5-2 kV; dry gas, 10 L/min; dry temperature, 230 °C. The nano-ESI source (Bruker Daltonik) was equipped with distal coated silica tips (FS360–20–10-D; New Objective). The system was operated with automatic switching between MS and MS/MS modes. The MS scanning was performed in the standard-enhanced resolution mode at a scan rate of 8100 m/z per second with an aimed ion charge control (ICC) of 200,000 in a maximal fill-time of 200 ms. A total of five scans (ranging from 300 to 1800 m/z) were averaged to obtain MS spectra. The three most intense and preferentially doubly charged ions, were selected on each MS spectrum for further isolation and fragmentation. Generally, MS/MS spectra were the sum of five scans ranging from 100 to 2200 m/z, at a scan rate of 26,000 (m/z)/s (ultrascan resolution mode). To generate fragment ions, low-energy CID was performed on previously isolated peptide ions by applying a fragmentation amplitude of 1.15 V. Exclusion limits were automatically placed on previously selected mass-to-charge ratios for 1 min. The ion trap instrument was externally calibrated with commercially available standard compounds.

Proteins were identified by searching the National Center for Biotechnology Information non-redundant database (NCBInr, www.ncbi.nlm.nih.gov) using the Mascot program (in-house version 2.2, Matrix Science, London, UK). The following parameters were adopted for database searches: complete carbamidomethylation of cysteines and partial oxidation of methionines, peptide mass tolerance ± 1.2 Da, fragment mass tolerance ± 0.9 Da, missed cleavages 2. For positive identification, the score of the result of (- 10 x Log(P)) had to be over the significance threshold level ($P<0.05$). In auto-MS/MS files, only peptides with Mascot scores greater than 30 were considered. Peptide fragmentation spectra were also verified manually. In this manual verification, the mass error, the presence of fragment ion series, and the expected prevalence of C-terminus containing (y-type) ions in the high mass range were all taken into account. All 2-DE spots required a minimum of two verified peptides to be identified. Moreover, replicate measurements (n = 3) confirmed the identity of these protein hits.

Information on protein biological and molecular function, as well as cellular compartment localization was harvested through bioinformatic approaches including both direct interrogation of the UniProt database and the FatiGO/Babelomics 4.2 tool.[14] Relevant ontologies (GO), Biocarta and KEGG pathways (*P*-value < 0.05 two-tailed Fisher's exact test of the submitted dataset against the rest of the human genome) are reported in the text and were used to catalogue functional protein groups in the Results and Discussion sections.

Oxidative stress

Reactive oxygen species

N,N-diethyl-para-phenylendiamine was dissolved in 0.1M sodium acetate buffer (pH 4.8) to obtain a final concentration of 100 g/mL (R1 solution as a chromogen). Ferrous sulfate was dissolved in 0.1M sodium acetate buffer (pH 4.8) to obtain a final concentration of 4.37 M (R2 solution as a transition-metal ion).

The hydrogen peroxide solution, at increasing dilutions, was used as a standard solution for generating a calibration curve. To process the reaction, 96-well microtiter plates (Nalge Nunc International, USA) were used. The Spectra Max Plus (Molecular Device Corp., USA) was used as a spectrophotometric plate reader. A volume of 5 μL of either hydrogen peroxide standard solution (for generating a calibration curve) or RBC lysate was added to 140 μL of 0.1M sodium acetate buffer (pH 4.8) in one well of a 96-well microtiter plate, which reached a temperature of 37 °C after 5 min. A volume of 100 μL of the mixed solution, which was prepared from R1 and R2 at a ratio of 1:25 before use, was added to each well as a starter. Then, after pre-incubation at 37 °C for 1 min using a spectrophotometric plate reader, absorbance at 505 nm was measured for a fixed time (between 60 and 180 s) at 15 s intervals. A calibration curve was automatically constructed from the slopes, which were calculated based on varying (delta) absorbance at 505 nm each time (min) corresponding to the concentration of hydrogen peroxide. ROS levels in RBC were calculated by the analyzer (spectrophotometric plate reader) from the calibration curve, and expressed as equivalent to levels of hydrogen peroxide (1 unit = 1.0 mg H_2O_2/L).

Carbonyl content

Protein oxidation of erythrocytes was assayed as protein carbonyl according to the method of Levine *et al.*[15] Proteins were precipitated from RBC lysates by addition of 10% trichloroacetic acid (TCA) and resuspended in 1.0 mL of 2 M HCl for blanks and 2 M HCl containing 2% 2,4- dinitrophenyl hydrazine. After incubation for 1 h at 37°C, protein samples were washed with alcohol and ethyl acetate, and re-precipitated by addition of 10% TCA. The precipitated protein was dissolved in 6 M guanidine hydrochloride solution and measured at 370 nm. Calculations were made using the molar extinction coefficient of $22\times10^3 M^{-1}$ cm^{-1} and expressed as nmol carbonyls formed per mg protein. Total protein in the RBC pellet was assayed according to the method of Lowry *et al.*[16] using bovine serum albumin as standard.

Red blood cell membrane lipoperoxidation

Malondialdehyde (MDA) levels were estimated in RBC using the method of Stocks and Dormand with some modifications.[17] Briefly, 0.2 mL of packed RBC were suspended in 3.0 mL of Krebs's Ringer phosphate buffer (KRBP) solution (pH 7.4) and 1 mL of the cell suspension was treated with 1 mL of 10% TCA and centrifuged at 1,000 × g for 5 min. Next, 1 mL of the supernatant was mixed with 1 mL of 0.67% thiobarbituric acid and heated over a water bath for 20 min at 85–90 °C. The solution was cooled and read against a complementary blank at 532 nm (OD1) and 600 nm (OD2). A blank was prepared separately without packed RBC. The net optical density (OD) was calculated after subtracting absorbance at OD2 from that at OD1. The MDA level was determined from the standard plot and expressed as nmol/mL of packed RBC.

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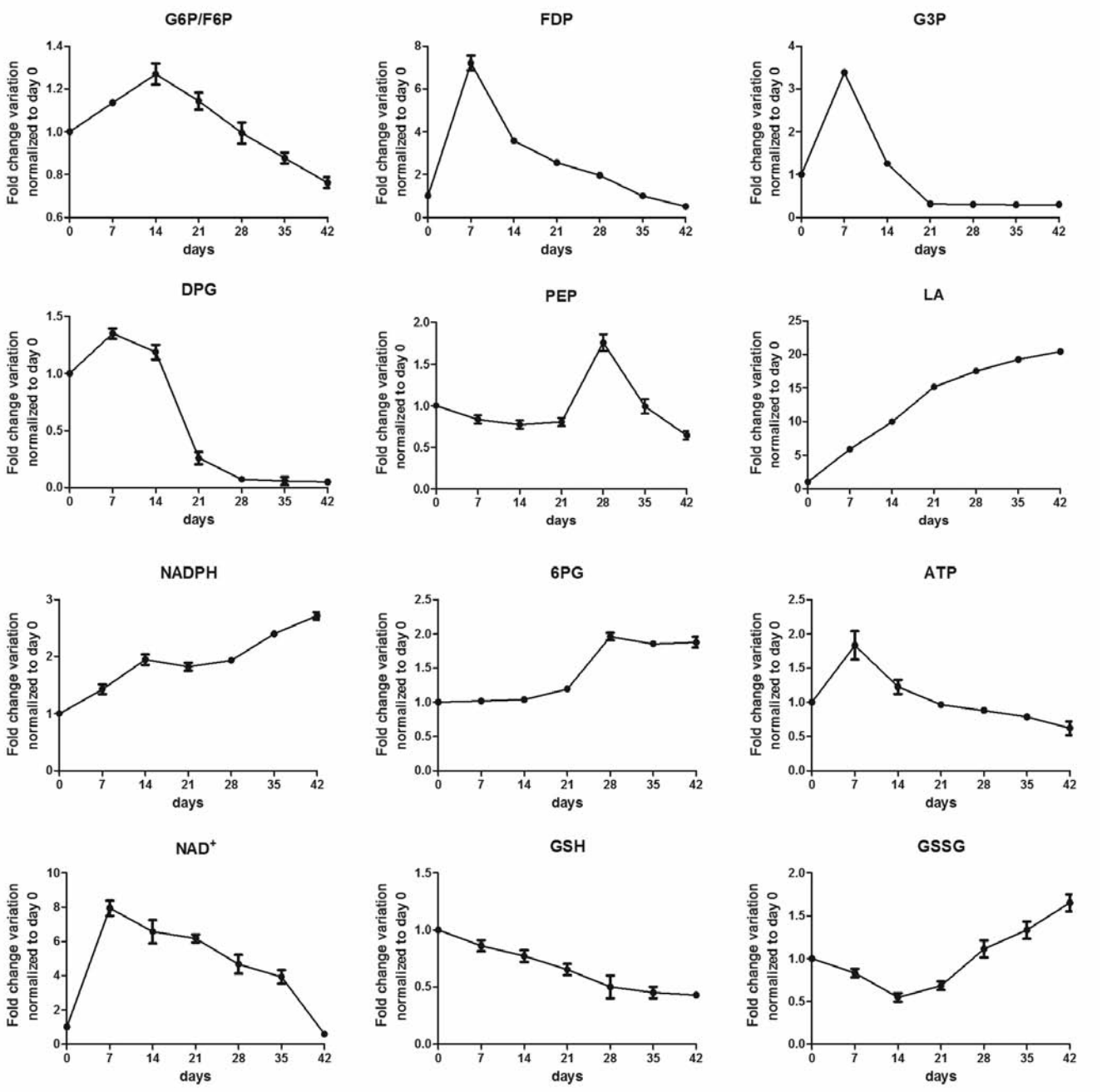
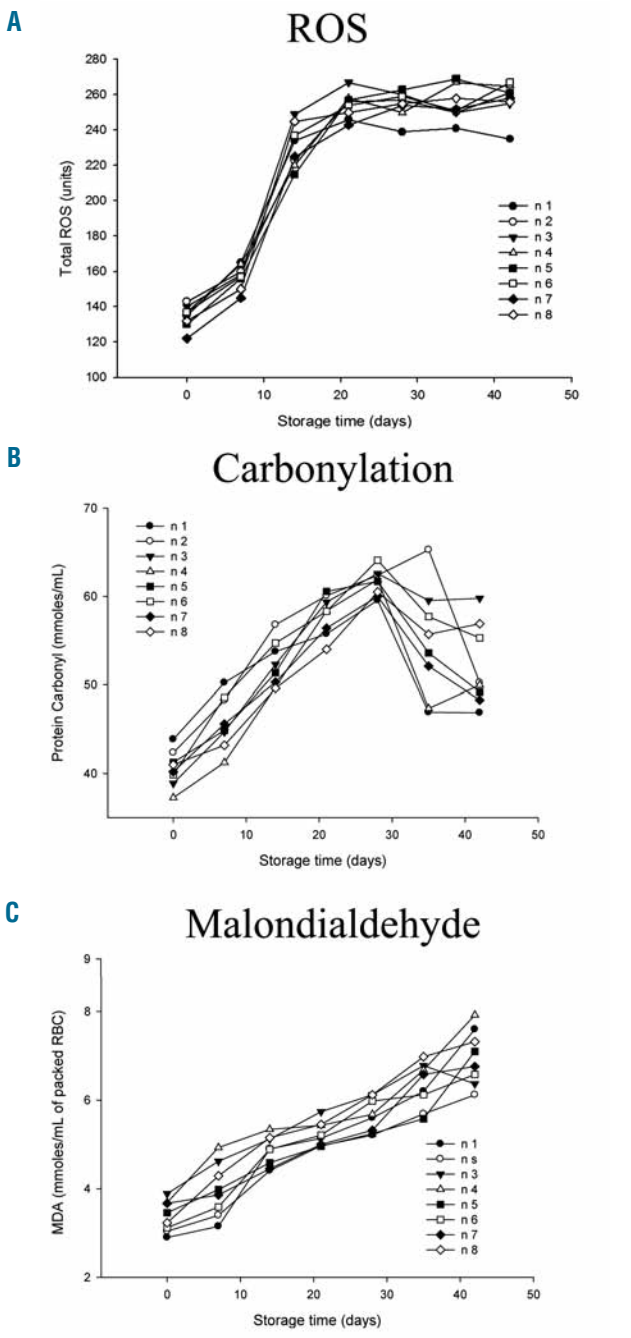


Online Supplementary Figure S1. Time course metabolomic analyses of SAGM-stored RBC, after normalization against day 0 control values, as in mass spectrometry-based metabolomics analysis on MAP-stored RBC.[20] G6P/F6P: glucose/fructose 6-phosphate; FDP: fructose 1,6 diphosphate; G3P: glyceraldehyde 3-phosphate; DPG: diphosphoglycerate; PEP: phosphoenolpyruvate; LA: lactate; NADPH: nicotinamide adenine dinucleotide phosphate; 6PG: 6-phosphogluconate; ATP: adenosine triphosphate; NAD^+: nicotinamide adenine dinucleotide; GSH: reduced glutathione; GSSG: oxidized glutathione.



Online Supplementary Figure S2. Levels of reactive oxygen species (ROS) (1 unit = 1.0 mg H_2O_2/L) over the whole duration of the storage for each tested unit (A). Protein carbonylation measurements (mmol/mL) over the whole duration of the storage for each tested unit (B). Malondialdehyde (MDA) measurements (mmol/mL) over the whole duration of the storage for each tested unit (C).

Online Supplementary Table S1. Protein spots showing statistically significant differential photodensities between day 0 and day 35 RBC membrane samples.

A. Extraction performed in the absence of NEM (days 0, 14 and 35)

SPOT	Mr, kDa theor.	Seq Cov (%)	NCBI Accession Number	Protein ID [Homo sapiens]
104	247026	9%	gi|338441	Beta-spectrin
176	282024	4%	gi|338438	Erythroid alpha spectrin
211	282024	4%	gi|338438	Erythroid alpha spectrin
226	11537	17%	gi|66473265	Beta globin chain
239	281039	15%	gi|115298659	Spectrin alpha chain, erythrocyte
249	6595	38%	gi|13492060	Truncated beta-globin
303	42623	21%	gi|14277739	Chain P, crystal structure of the cytoplasmic domain of human erythrocyte band-3 protein
320	66756	3%	gi|4758274	Protein 4.1 isoform 6
327	92774	4%	gi|62088878	Protein 4.1 variant
342	92774	4%	gi|62088878	Protein 4.1 variant
402	22049	40%	gi|32189392	Peroxiredoxin 2 isoform a
417	22049	40%	gi|32189392	Peroxiredoxin 2 isoform a
389	22049	40%	gi|32189392	Peroxiredoxin 2 isoform a
187	28876	36%	gi|5453990	Proteasome activator subunit 1 isoform 1

B. Extraction performed in the presence of NEM (days 0 and 35).

SPOT	Mr, kDa theor.	Seq Cov (%)	NCBI Accession Number	Protein ID [Homo sapiens]
42	73518	24%	gi|4557625	Glutamate—cysteine ligase catalytic subunit isoform a
55	63227	15%	gi|5803181	Stress-induced-phosphoprotein 1
93	52928	34%	gi|16306550	Selenium-binding protein 1
97	57794	48%	gi|5453603	T-complex protein 1 subunit beta isoform 1
	52492	4%	gi|4505237	55 KDa erythrocyte membrane protein isoform 1 (ankyrin 1)
98	52928	25%	gi|16306550	Selenium-binding protein 1
	59035	21%	gi|1136741	KIAA0002
101	60981	17%	gi|37787305	Nicotinate phosphoribosyltransferase-like protein
	52057	16%	gi|4503729	Peptidyl-prolyl cis-trans isomerase FKBP4
104	247026	9%	gi|338441	Beta-spectrin
118	42938	26%	gi|13277550	FLOT2 protein
176	282024	4%	gi|338438	Erythroid alpha spectrin
211	47055	32%	gi|33186798	DNA-damage inducible protein 2
	41477	25%	gi|19923193	Hsc70-interacting protein
226	35325	24%	gi|4506127	Ribose-phosphate pyrophosphokinase 1
	11537	17%	gi|66473265	Beta globin chain
233	31951	23%	gi|6841176	HSPC263
	32630	5%	gi|4759274	Thioredoxin-like protein 1
239	281039	15%	gi|115298659	Spectrin alpha chain, erythrocyte
242	33681	45%	gi|3929617	Alpha SNAP
248	27249	59%	gi|4588526	Nuclear chloride cannel
	31050	10%	gi|504011	Glutamate—cysteine ligaase regulatory subunit
249	31956	68%	gi|33413400	S-formylglutathione hydrolase
	6595	38%	gi|13492060	Truncated beta-globin
253	31860	46%	gi|181184	Stomatin peptide
254	29243	61%	gi|24119203	Tropomyosin alpha-3 chain isoform 2
263	27815	58%	gi|6912586	6-Phosphogluconolactonase
	27833	48%	gi|4758484	Glutathione S-transferase omega-1 isoform 1
270	33667	32%	gi|47933379	Alpha-soluble NSF attachment protein
274	26337	50%	gi|5174741	Ubiquitin carboxyl-terminal hydrolase isozyme L3
	27899	21%	gi|4507953	14-3-3 Protein zeta/delta
280	21960	52%	gi|544759	Biliverdin-IX beta reductase isozyme I
281	21960	35%	gi|544759	Biliverdin-IX beta reductase isozyme I

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292	27833	48%	gil4758484	Glutathione S-transferase omega-1 isoform 1
	27704	47%	gil312597295	Chain A, crystal structure oh human glutathione transferase omega 1, delta 155
295	27815	48%	gil6912586	6-Phosphogluconolactonase
297	22178	46%	gil577777	Glutathione peroxidase
	21699	37%	gil119623103	Proteasome 26S subunit, non ATPase, 10, isoform
303	42623	21%	gil14277739	Chain P, crystal structure of the cytoplasmic domain of human erythrocyte band-3 protein
320	96646	26%	gil6424942	ALG-2 interacting protein 1
	66756	3%	gil4758274	Protein 4.1 isoform 6