Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 39 (2005) 636–642
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Human red blood cells targeted metabolome analysis of glycolysis cycle metabolites by capillary electrophoresis using an indirect photometric detection method

Michał J. Markuszewski *, Marta Szczykowska, Danuta Siluk, Roman Kaliszan

Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk, Gen. J. Hallera 107, 80-416 Gdańsk, Poland

Received 15 March 2005; accepted 15 April 2005
Available online 31 May 2005

Abstract

A capillary electrophoretic (CE) method with UV indirect spectrophotometric detection for determination of the main compounds of glycolysis in human erythrocytes has been elaborated. Blood samples for electrophoretic analysis were prepared by isolation of erythrocytes, lysis of the cells by heating in double-distilled water and subsequent ultrafiltration with a centrifuge equipped with filter devices: M_r cut off 5000. Using 20 mM 2,6-pyridinedicarboxylic acid (PDC) as a highly UV absorbing carrier electrolyte and 4 mM cetyltrimetylammonium bromide (CTAB), at the resulting pH 12.3, nine of the glycolysis intermediates were separated and characterized. The repeatability and linearity of the method was assessed with percent relative standard deviation (%R.S.D.) for migration time ranging from 0.3 to 1.9% and correlation coefficients of 0.991–0.999 for the studied concentration range. Limits of detection (LOD) for the analyzed metabolites were in the range of 6.25×10^{-6} to 5.0×10^{-5} M. The optimized CE method was used to compare metabolome content of red blood cells of 22 healthy volunteers. Mean metabolite concentrations in erythrocytes ranged from 49.6 μM for fructose-6-phosphate (F-6-P) to 3.1 mM for 2,3-diphospho-D-glyceric acid (2,3-DPG). The method can be readily applied in clinical, pathophysiological and epidemiological studies.

Keywords: Metabolome analysis; Erythrocytes; Glycolysis; Capillary electrophoresis; Indirect UV detection method

1. Introduction

Metabolome, by analogy to the genome or proteome, is defined as the complete set of metabolites synthesized by a given biological system, such as organism, organ, tissue, cell or cell compartment. After spectacular advances of genomics and proteomics, metabolome analysis seems to be the next emerging major research field in bioscience. Metabolome analysis can be described as a comprehensive, qualitative and quantitative analysis of all low molecular mass compounds present in living cell.

Metabolites are the final products of cellular regulatory processes, and their quantitative levels can be regarded as the ultimate response of biological systems to genetic and environmental changes [1]. Data obtained from metabolome analysis can be used for various aims like: simulation of the biological activity with genes coded in genome; studying of functions of new genes and effects of genes mutation on metabolites level; production of valuable metabolites by gene technology [1]. Recently, usefulness of capillary electrophoresis in metabolomic studies for investigation of environmental influences on concentrations of carboxylic acid metabolites from Krebs cycle [2] and nucleotide metabolites in *Bacillus subtilis* bacteria cell extracts [3,4] was demonstrated.

Human erythrocytes are peculiar type of cells present in an organism that are void of specific organelles, like nucleus and mitochondria. Energy necessary for proper functioning of these cells is almost exclusively derived from glycolysis. During glycolysis cycle, glucose in cascade of reactions is converted to lactate or (in anaerobic cells) to ethanol.

In routine analysis, glycolysis metabolites are normally determined by enzymatic methods with photometric detec-

* Corresponding author. Tel.: +48 58 3493260; fax: +48 58 3493262.
E-mail address: markusz@amg.gda.pl (M.J. Markuszewski).

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.04.015

tion [5]. However, enzymatic methods allow only a single metabolite determination during each analysis. Recently, an isotachophoretic method has been established for determination of the main compounds of glycolysis in human erythrocytes. Renner et al. [6] analyzed influence of different glucose concentrations on glycolysis metabolic pathway in order to model the situation occurring in diabetes. CE with indirect fluorescent detection was used for the analysis of lactate and pyruvate metabolites in the single erythrocytes [7]. Also, adenine nucleotides engaged in glycolysis metabolic pathway in human erythrocytes were measured with CE [8].

Nowadays, capillary electrophoresis–electrospray ionization mass spectrometry (CE–ESI-MS) became a powerful analytical tool widely used for bioanalytical applications. Soga et al. [9,10] developed a sensitive, selective and rapid CE–ESI-MS method for the analysis of anionic species. The method was applied to the comprehensive analysis of intracellular metabolites of glycolysis and tricarboxylic acid cycle pathways extracted from *B. subtilis* bacteria [9]. CE–ESI-MS method demonstrated also its utility for monitoring changes of glycolysis metabolites during bacteria *B. subtilis* sporulation [11].

Hyphenation of CE with MS detection provides powerful analytical technique. CE allows fast analysis with efficient resolution meanwhile MS detects analytes with high selectivity and sensitivity. However, due to the complex and expensive instrumentation that requires hyphenation of both techniques, the indirect UV detection method is still of strong interest among many analytical and clinical laboratories. Although indirect UV method is not as selective and sensitive as MS method it possess advantages of simplified analytical procedures as well as less sophisticated equipment resulting in lower costs of analysis.

The aim of presented studies was to determine the most important intermediates of glucose metabolism in erythrocytes in a single electrophoretic run to get a convenient tool for metabolic survey of the glycolysis cycle in such disorders like, e.g. anemia and glucose-6-phosphatase deficiency. The optimized analytical conditions were to be applied to determine erythrocyte content in healthy volunteers in order to establish the metabolites' concentration ranges for comparative biomedical purposes.

2. Materials and methods

2.1. Apparatus

All the experiments were performed with a P/ACE MDQ capillary electrophoresis system (Beckman Instruments, Fullerton, CA, USA) equipped with a PDA detector. For the separation of analytes, bare fused silica capillaries, 100 cm total length (90 cm effective length), 50 μm i.d. × 365 μm o.d. (Beckman Instruments, Fullerton, CA, USA) were used. PDA detection wavelength was set on 214 nm in indirect mode. The capillary, as well as sample compartment, temperature was maintained at 15 °C. Samples were injected by hydrodynamic mode, 3447.4 Pa × 20 s. During separation run a constant voltage of 25 kV was applied at a negative polarity (cathode at the injection end). Observed electric current at above described electrophoretic conditions was ∼50 μA.

Prior to first use, new capillaries were rinsed with 1.0 M HCl (137.9 kPa × 15 min), then with 0.1 M NaOH (137.9 kPa × 30 min) and deionized water (137.9 kPa × 10 min), and finally with the background electrolyte (BGE) (137.9 kPa × 30 min). Each day before the analysis started, capillaries were pretreated with deionized water for 10 min (137.9 kPa) and BGE for 30 min (137.9 kPa). For conditioning, each separation run was preceded by and ended with a 2 min (137.9 kPa) rinse with the background electrolyte.

2.2. Reagents and samples

β-D-Glucose-6-phosphate, monosodium salt (G-6-P); D-fructose-6-phosphate, disodium salt (F-6-P); D-fructose-1,6-diphosphate, sodium salt (FDP); dihydroxyacetone phosphate, dilithium salt (DAP); 2,3-diphospho-D-glyceric acid, pentacyclohexylammonium salt (2,3-DPG); D-(−)3-phosphoglyceric acid, disodium salt (3-PG); phospho(enol)pyruvate, monopotassium salt (PEP), 2,6-pyridinedicarboxylic acid (PDC), tetradecyltrimethylammonium bromide (TTAB) and dodecyltrimethylammonium bromide (DTAB) were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Pyruvic acid, sodium salt (PYR); cetyltrimethylammonium bromide (CTAB); sodium hydroxide (NaOH) were from Fluka Chemie (Buchs, Switzerland). L-Lactic acid (LAC) was purchased from Aldrich Chem. Co. (St. Louis, USA). All used reagents were of analytical-reagent grade. Water was purified with a Milli-Q system (Millipore, Bedford, MA, USA).

A sample stock solution of each metabolite from glycolysis pathway (G-6-P, F-6-P, FDP, DAP, 2,3-DPG, 3-PG, PEP, PYR and LAC) was prepared in purified water at the concentration of 20 mM and stored at −20 °C. Prior to analysis, fresh metabolites standard solutions were prepared by dilution with water to obtain appropriate final concentration. Chemical structures of the analyzed metabolites from glycolysis cycle are presented on Fig. 1.

Optimized background electrolyte (BGE) consisted of 20 mM PDC as a strongly UV absorbing carrier electrolyte and 4 mM CTAB used to reverse electroosmotic flow (EOF) and shorten analysis time. Background electrolyte pH was adjusted with 1 M NaOH to 12.3. Strongly basic pH of BGE was crucial for ensuring ionization of fructose and glucose sugar hydroxyl groups, which pKa values were equal and above 12 (fructose $pK_a = 12.03$, glucose $pK_a = 12.28$). BGE solutions prior to use were sonicated and filtered through 0.45 μm membrane filters. During initial experiments spiking sample solutions with the known standard did the peak identification for individual analyte. When extracted blood samples were analyzed, the peak identification was based on

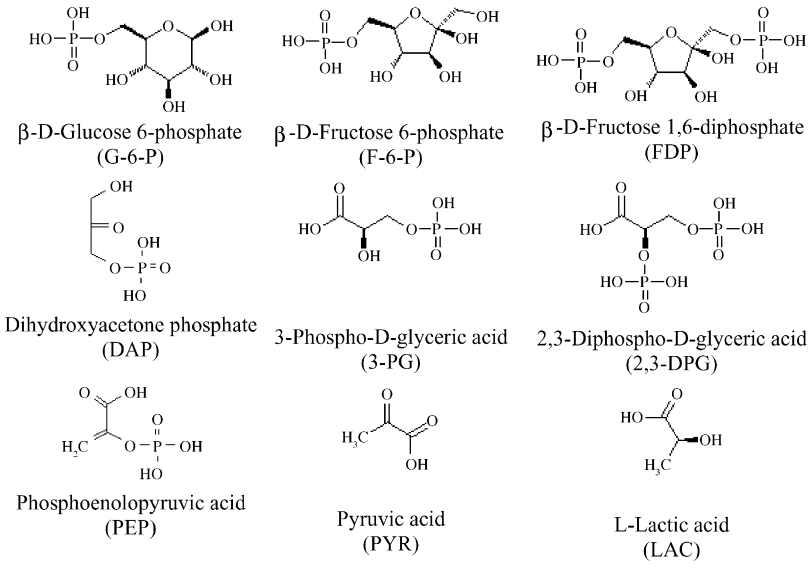

Fig. 1. Structural formulas of glycolysis metabolites studied.

the migration time and spectral profile collected with help of PDA detector and the performance of spectral library search of previously created database.

2.3. Blood samples preparation

Healthy male volunteers, who gave their informed consent, donated blood samples used for measurements of glycolysis metabolites concentration in erythrocytes. Blood sample preparation and extraction of erythrocyte were done according to Renner et al. [6] with modifications. Blood was collected by venipuncture into 3.2 % sodium citrate solution (volume ratio 9:1, v/v) and centrifuged at 90 g for 7 min at room temperature when blood platelets had been removed. The supernatant was discarded and blood was centrifuged again at 1430 g for 10 min. That way obtained erythrocyte suspension was washed with 0.9% NaCl solution and further centrifuged at 1430 g for 3 min. Supernatant was withdrawn and the same washing procedure with 0.9% NaCl solution was repeated twice. Finally, 500 μl of erythrocytes were transferred to a clean tube, suspended in one volume of double-distilled, deionized water and then boiled for 2 min. After centrifugation at 1430 g for 3 min, the supernatant was ultrafiltered with centrifuge filter devices, M_r cut off 5000 (Ultrafree Centrifugal Filter Units, Millipore, Bedford, MA, USA), at 15100 g for 20 min. The finally obtained filtrate was subjected to capillary electrophoresis experiments.

3. Results and discussion

Optimum performance of CE analysis with indirect UV detection requires a proper selection of carrier electrolyte [12]. Mobilities of co-ions of the BGE influence the electromigration dispersion of the zones of analytes. According to the simplified rule, the BGE co-ions mobility matching those of the majority of the analytes would give best separation and resolution due to peak symmetry. However, electromigration dispersion, which affects peak shape and thus separation, is not a simple function of matching of mobilities of sample ions and BGE co-ions [13,14]. The carrier electrolyte should also possess a high molar absorptivity which is a key parameter influencing the method sensitivity. The molar absorptivity of PDC (ca. 43500 l mol^{-1} cm^{-1}) is one of the highest of all the available carrier electrolytes and its electrophoretic mobility matches close that of the analyzed carboxylic acids [15].

During preliminary experiments, the influence of BGE concentration, pH and the type of EOF modifiers on the separation of glycolysis metabolites was investigated. Studies were made in the BGE concentration range from 0.5 mM to 40 mM of PDC and in pH range from 11.3 to 12.3. The PDC was selected as the BGE since it provided high buffering capacity for organic anions and carbohydrate analysis in a broad range of pH [15–17]. At the pH range 5–7, most appropriate pH due to the PDC pK_a value, F-6-P and G-6-P were not separated. Shen et al. [18] observed full separation of those two isomers at very alkaline pH >11. In our experiments, when pH of BGE was elevated above 12, we also have succeeded in baseline separation of F-6-P and G-6-P peaks. This was due to the difference in dissociation of fructose and glucose sugar hydroxyl groups (fructose pK_a = 12.03, glucose pK_a = 12.28) [16].

For a rapid separation of metabolite anions, cationic surfactants were included in the BGE to reverse the EOF. Appropriate choice of EOF modifier is essential to avoid interference from system peaks when indirect detection is being used. The effects of three cationic surfactants, namely, cetyltrimethylammonium bromide (CTAB), tetradecyltrimethylammonium bromide (TTAB) and dodecyltrimethylammonium bromide (DTAB) on the separation

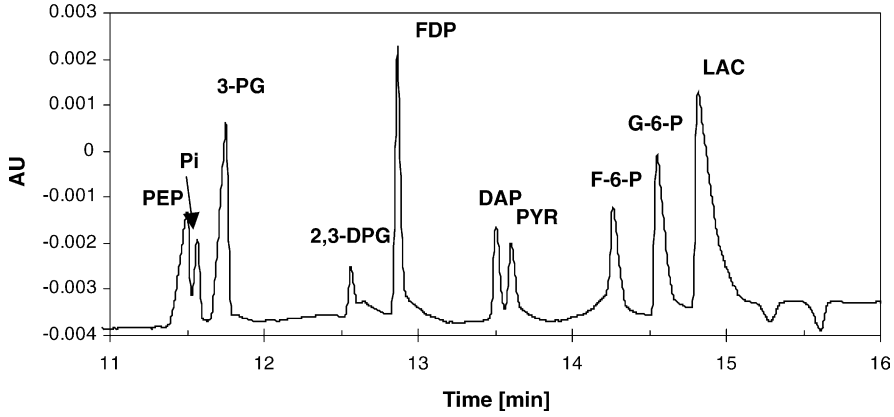

Fig. 2. Electropherogram of glycolysis cycle metabolites from standard mixture. Identified peaks: phosphoenolpyruvate, PEP; inorganic phosphate, P_i; 3-phosphoglyceric acid, 3-PG; 2,3-diphosphoglyceric acid, 2,3-DPG; fructose-1,6-diphosphate, FDP; dihydroxyacetone phosphate, DAP; pyruvic acid, PYR; fructose-6-phosphate, F-6-P; glucose-6-phosphate, G-6-P; lactic acid, LAC. The analyzed glycolysis metabolites were in following concentrations: PEP, 2.25×10^{-5} M; 3-PG, 4.5×10^{-5} M; 2,3-DPG, 7.5×10^{-5} M; FDP, 9×10^{-5} M; DAP, 7.5×10^{-5} M; PYR, 9×10^{-5} M; F-6-P, 1.2×10^{-4} M; G-6-P, 5.25×10^{-5} M; LAC, 8×10^{-5} M. Experimental conditions are as follow: BGE consists of 20 mmol/l PDC and 4 mmol/l CTAB, pH 12.3; bare fused silica capillary, 100 cm total length (90 cm effective length), 50 μm i.d. × 365 μm o.d.; detection at 214 nm; capillary and sample compartment temperature 15 °C; hydrodynamic injection mode, 3447.4 Pa × 20 s; voltage 25 kV.

selectivity of metabolite anions were investigated. The most appropriate EOF modifier, by means of separation selectivity, CTAB cationic surfactant was found. For the reversal of EOF 0.5 mM concentration of CTAB is sufficient. However, the concentration of cationic surfactant in the BGE also influences selectivity [19]. CTAB concentrations studied were in the range from 0.5 mM to 10 mM. The selectivity changes observed with increased CTAB concentration attributed to a combination of ion-pairing and ion-exchange effects [19]. At concentration above the critical micelle concentration (CMC), chromatographic partitioning between analyte and micelle occurs. CMC in case of CTAB is equal to 0.9 mM [20]. Finally, in the BGE, as the most appropriate by means of separation selectivity, 4 mM concentration of CTAB was used.

In BGE of pH 12.3 consisting of 20 mM PDC and 4 mM CTAB, a baseline level resolution of nine glycolysis metabolites was achieved (Fig. 2). The migration order of glycolysis metabolites is dependent upon the mobility of anions. Similarly, peak shape is influenced by mismatches in mobilities of analyte and the BGE co-ions that results in disturbed concentration distribution. Analytes that possess higher mobility than the BGE co-ions migrates with a concentration distribution diffused at the front and sharp at the rear of the band, resulting in a fronting peak. Tailing peaks are when analytes migrate with lower mobility than the BGE co-ions and a concentration distribution band is diffuse at the rear of the zone and sharp at the front. When the mobility of analyte and BGE co-ions are approximately the same symmetrical peaks appear [21].

The proposed CE method with indirect photometric detection was checked for precision, linearity and limit of detections. Precision of the method was assessed by calculating percent relative standard deviations (%R.S.D.) for migration times, peak areas and the corrected peak areas (Table 1) in standard mixture of analytes, each presented at concentration 400 μM, by six replicate injections ($n = 6$) during a single day (intra-day precision) and in three consecutive days (inter-day precision).

The %R.S.D. for the migration times of eight glycolysis metabolites from six consecutive injections ($n = 6$) within one day are in the range from 0.3 to 1.9%. The %R.S.D. values for the peak area and for the corrected peak area are in the range of 2.0–10.4 and 1.9–10.3%, respectively. Concentration of sample during repeatability studies was 400 μM and CE analyses were performed with the hydrodynamic injection (3447.4 Pa × 20 s).

As expected, the day-to-day repeatability of migration times was lower than that determined during one day and ranged from 3.8 to 5.1%. The %R.S.D. values for the peak area measured day-to-day ranged from 10.2 to 15.8%. Probably, changes of the capillary inner surface conditions, variability of BGE preparation and usage of unbuffered BGE affected the inter-day repeatability of migration times and peak areas.

Table 1
Repeatability of migration time (t_M), peak area (P_{Area}) and corrected peak area (CP_{Area}) as expressed by percent relative standard deviation (% R.S.D.)

Metabolite	% R.S.D. ($n = 6$)				
	t_M		P_{Area}		CP_{Area} Intra-day
	Intra-day	Inter-day	Intra-day	Inter-day	
PEP	0.3	3.8	6.8	13.0	6.6
3-PG	1.5	3.9	4.6	12.3	3.6
2,3-DPG	1.3	4.0	2.0	10.2	1.9
FDP	1.6	4.2	8.3	15.8	7.0
DAP	0.6	4.5	7.8	14.0	7.8
PYR	0.6	4.7	10.4	15.6	10.3
F-6-P	1.9	5.0	8.3	13.3	7.2
G-6-P	0.6	5.1	3.8	11.5	3.8

Table 2
Linearity range (μM), calibration line equation and detection limits determined for analyzed glycolysis cycle metabolites

Metabolite	Linearity range (μM)	Calibration line[a] ($n = 6$)		Detection limits ($S/N = 3$)	
		$y = ax + b$	R	LOD (μM)	LOD (μg/ml)
PEP	12.5–400	$y = 28x - 131$	0.999	6.25	1.29
3-PG	12.5–400	$y = 44x - 266$	0.998	6.25	1.44
2,3-DPG	125–2000	$y = 53x - 6564$	0.991	50	38.1
FDP	12.5–400	$y = 41x - 379$	0.999	6.25	2.13
DAP	50–400	$y = 13x + 92$	0.997	25	4.25
PYR	50–400	$y = 12x - 236$	0.997	25	2.75
F-6-P	25–400	$y = 26x - 486$	0.997	12.5	3.80
G-6-P	12.5–400	$y = 37x - 171$	0.999	6.25	1.76

R is correlation coefficient of linear equation. Number of replicates performed at each concentration, $n = 6$.
[a] Calibration line: concentration = slope × peak area + intercept.

The linearity of the method was viewed as dependence of the individual metabolite's peak area on its concentration. The linearity was investigated by analyzing standard solutions containing a mixture of metabolites at six known concentrations ranging typically from 12.5 to 400 μM. The only significant exception was 2,3-DPG for which linearity range studied was from 125 to 2000 μM because of its high physiological concentration level (∼4000 μM) in human erythrocytes. The calibration curves were established from the dependence of the individual metabolite's peak area on its concentration. The newly elaborated CE method appeared to be linear over a wide concentration range (Table 2). Obtained correlation coefficients ranged from 0.997 to 0.999 (excepting 2,3-DPG, for which $R^2 = 0.991$).

The limit of detection (LOD) for a non-absorbing analyte detected with indirect UV detection method is given by [21]:

$$C_{\mathrm{lod}} = \frac{C_{\mathrm{p}}}{\mathrm{TR} \times D_{\mathrm{r}}} = \frac{N_{\mathrm{BL}}}{\mathrm{TR} \times \varepsilon l}$$

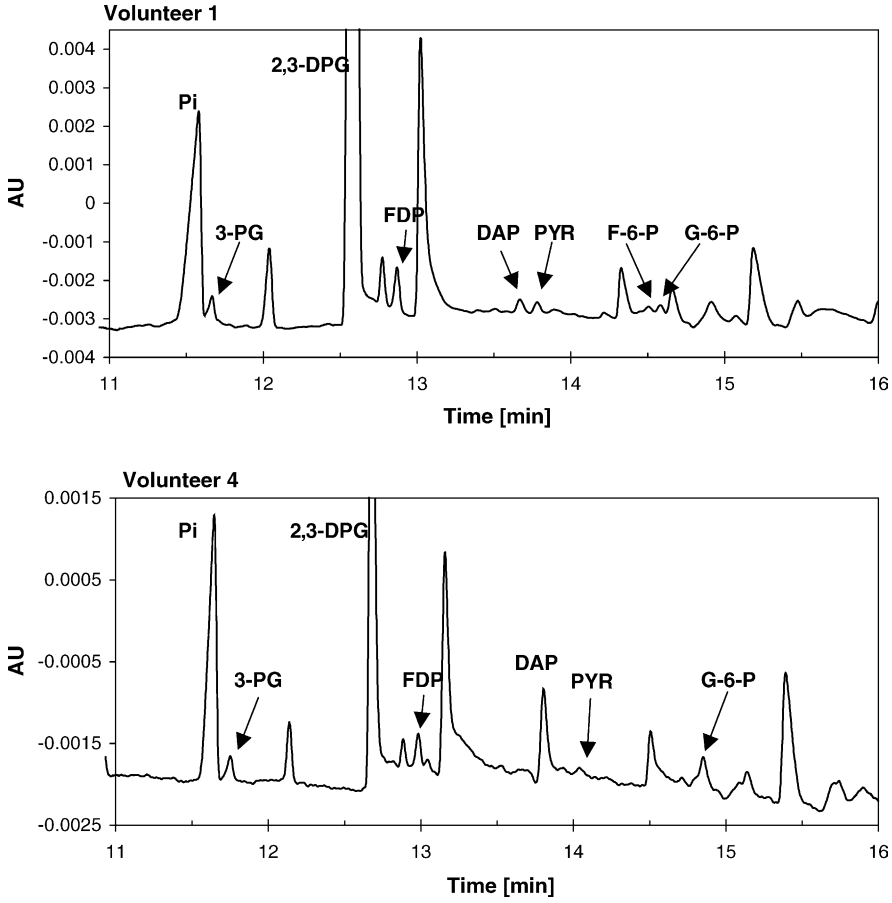

Fig. 3. Electropherograms of glycolysis cycle metabolites after extraction from human red blood cells donated by exemplary two volunteers. Identified peaks and experimental conditions see Fig. 2. The concentrations corresponding to the peak areas are given in Table 3.

Table 3
Average concentrations in μM of glycolysis metabolites and reference values from the literature [22–24]

	Mean concentration [μM]						
	3-PG (n = 22)	2,3-DPG (n = 22)	FDP (n = 22)	DAP (n = 13)	PYR (n = 17)	F-6-P (n = 14)	G-6-P (n = 19)
Mean [μM]	56.6	3158.5	112.2	221.3	133.9	49.6	136.0
S.D.	17	925	40	129	106	23	73
Reference values [22]	118.0	4 000	31.00	138.00	51.00	14.00	83.00
Reference values [23]	55–68.5	4170–5700	2–30	7.6–35		6–15.7	20–110
Reference values [24]	45	4171	2	9		9	28

S.D. denotes standard deviation and n number of determined cases.

where C_{lod} is the concentration limit of detection of non-absorbing analyte, C_p the concentration of the carrier electrolyte, TR the transfer ratio (i.e., the number of moles of the carrier electrolyte displaced by one mole of the analyte), D_r the dynamic reserve explained as the ratio of the background absorbance to the noise, N_{BL} the baseline noise, ε the molar absorptivity of the carrier electrolyte and l relates to the pathlength of the detection cell [21]. Generally, minimizing C_{lod} requires maximizing D_r by either reducing the noise or increasing the BGE background absorbance. The LODs for carbohydrate metabolites were in the range from 6.25 to 50 μM (1.29–38.1 μg/ml) with hydrodynamic injection (3447.4 Pa for 20 s), at a signal to noise ratio above three (Table 2).

The optimized indirect UV detection CE method has been applied to the analysis of metabolites from human blood erythrocytes (Fig. 3). Erythrocytes were previously extracted and lyzed as described in Materials and methods section. Seven metabolites from glycolysis metabolic pathway have been identified on electropherograms: 3-PG, 2,3-DPG, FDP, DAP, PYR, F-6-P and G-6-P.

Apart from several unidentified peaks present on electropherograms, there was also one large peak of inorganic phosphate (P_i). It is known from the literature [22] that the concentration of inorganic phosphate in red blood cell attains the level of 1 mM. Perhaps this is a reason why the peak of PEP could not be detected as covered by a peak of P_i, being at concentration about 50 times higher than PEP.

On erythrocyte electropherograms 2,3-DPG arises as the biggest peak. Again, it is not surprising in view of red blood cell metabolism specificity. In erythrocytes very important is Rapoport–Luebering cycle that metabolizes 1,3-diphosphoglycerate (1,3-DPG) into 2,3-DPG. 2,3-DPG is an important metabolite that plays regulatory role of oxygen transport. It affects affinity of hemoglobin to the oxygen and facilitates oxygen release from connection with oxyhemoglobin. In contradiction to most of the other cells, where are present only traces of 2,3-DPG, in erythrocytes the concentration of 2,3-DPG is the highest and reaches the level of about 4 mM.

Mean concentrations of glycolysis metabolites in erythrocytes collected from 22 healthy volunteers, determined by the indirect capillary electrophoresis method, are presented in Table 3. Reference values [22–24] were obtained with immunoassay methods. In case of blood erythrocytes of some volunteers, DAP and F-6-P were below measurable concentrations established by our method. However, LOD of F-6-P, 12.5 μM, is on a physiological level reported by other authors [22–24]. Only with elevated concentrations, above LOD, it was possible to quantify amounts of F-6-P.

Noted in the literature concentrations of DAP differ significantly between each other and range from 7.6 to 35 μM [23] and 9 μM [24] to 138 μM [22]. Results of our measurements were in compliance with those phenomena with measured DAP mean concentration of 221 (±129) μM.

For the remaining metabolites determined mean concentrations are similar to that reported by other authors and measured with immunoassay methods.

4. Conclusions

The established new CE method with indirect UV detection allows determination of seven main glycolysis metabolites in less than 15 min in a single electrophoretic run. That is a meaningful advantage over the usually used immunoassay approach that allows only single metabolite determination. The method can be readily applied to metabolomic studies of specific disease states.

Acknowledgements

The project was supported by the Komitet Badań Naukowych, Warsaw, Poland (Grant KBN No. 3PO5F 027 24).

References

[1] S. Terabe, M.J. Markuszewski, N. Inoue, K. Otsuka, T. Nishioka, Pure Appl. Chem. 73 (2001) 1563–1572.
[2] M.J. Markuszewski, K. Otsuka, S. Terabe, K. Matsuda, T. Nishioka, J. Chromatogr. A 1010 (2003) 113–121.
[3] P. Britz-McKibbin, M.J. Markuszewski, T. Iyanagi, K. Matsuda, T. Nishioka, S. Terabe, Anal. Biochem. 313 (2003) 89–96.
[4] M.J. Markuszewski, P. Britz-McKibbin, S. Terabe, K. Matsuda, T. Nishioka, J. Chromatogr. A 989 (2003) 293–301.
[5] E. Beutler, Red Cell Metabolism. A Manual of Biochemical Methods, third ed., Grune & Stratton Inc., Orlando, 1984.
[6] S. Renner, V. Prohaska, C. Gerber, D. Niethammer, G. Bruchelt, J. Chromatogr. A 919 (2001) 247–253.

[7] Q. Xue, E.S. Yeung, J. Chromatogr. A 661 (1994) 287–295.
[8] N. Ozer, Y. Aksoy, I.H. Ogus, J. Biochem. Biophys. Methods 45 (2000) 141–146.
[9] T. Soga, Y. Ueno, H. Naraoka, Y. Ohashi, M. Tomita, T. Nishioka, Anal. Chem. 74 (2002) 2233–2239.
[10] K. Matsuda, H. Yamaguchi, Y. Ueno, T. Soga, Y. Fujita, T. Nishioka, Gen. Inform. 13 (2002) 593–594.
[11] T. Soga, Y. Ohashi, Y. Ueno, H. Naraoka, M. Tomita, T. Nishioka, J. Proteome. Res. 2 (2003) 488–494.
[12] H. Poppe, X. Xu, in: M.G. Khaledi (Ed.), High Performance Capillary Electrophoresis, Wiley, New York, 1998, p. 375.
[13] P. Gebauer, P. Bocek, Anal. Chem. 69 (1997) 1557–1563.
[14] P. Gebauer, P. Borecka, P. Bocek, Anal. Chem. 70 (1998) 3397–3406.
[15] T. Soga, G.A. Ross, J. Chromatogr. A 767 (1997) 223–233.
[16] T. Soga, D.N. Heiger, Anal. Biochem. 261 (1998) 73–78.
[17] T. Soga, G.A. Ross, J. Chromatogr. A 837 (1999) 231–239.
[18] P. Shen, D. Hauri, J. Ross, P.J. Oefner, J. Cap. Elec. 3 (1996) 155–163.
[19] A. Harakuwe, P.R. Haddad, TrAC 20 (2001) 375–385.
[20] W.L. Hinze, D.W. Armstrong, ACS Symposium Series, vol. 342, 1987.
[21] P. Doble, P.R. Haddad, J. Chromatogr. A 834 (1999) 189–212.
[22] S. Minakami, H. Yoshikawa, Biochem. Biophys. Res. Comm. 18 (1965) 345–349.
[23] M.V. Martinov, A.G. Plotnikov, V.M. Vitvitsky, F.I. Ataullakhanov, Biochim. Biophys. Acta 1457 (2000) 75–87.
[24] J.W. Harvey, in: J.J. Kaneko, J.W. Harvey, M.L. Bruss (Eds.), Clinical Biochemistry of Domestic Animals, Academic Press, San Diego, 1997, pp. 157–203.