Alterations of Cell Metabolome in G6PD Deficient Cells upon Oxidative Stress

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Abstract
Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most common genetic disorder of erythrocytes that affects more than 400 million individuals worldwide. G6PD plays an important role in regeneration of reduced nicotinamide adenine dinucleotide phosphate (NADPH) to maintain cellular redox homeostasis. Metabolomics approach provides comprehensive information such as biochemical reactions, metabolite networks, and their interaction in biological systems. This study used a LC-MS-based metabolomics approach to analyze the metabolites of G6PD-deficient erythrocytes under oxidative stress. Results showed that there were few differences in the global metabolic profile between normal and G6PD deficient erythrocytes under baseline condition. However, upon diamide treatment, metabolites such as glutathione metabolism like glutathione, oxidize glutathione, gamma-glutamylcysteine, glutamine, ophthalmate and nucleotide metabolism in G6PD deficient erythrocytes were significantly affected. Elevation of ophthalmate indicated the depletion of GSH and increased oxidative stress in G6PD deficient RBCs. Depletion of ATP and elevation of nucleotide metabolism reflected that G6PD-deficient RBCs were more energy demanding. Upon diamide treatment, glucose 6-phosphate, the substrate of G6PD, significantly increased in G6PD-deficient RBCs. In addition, diamide treatment induced high-molecular weight protein aggregates and decreased erythrocyte deformability. Taken together, these data indicate that LC-MS metabolomics based approach is useful to explore the relationship between altered RBC metabolism and cellular function under oxidative stress.

Experimental Design

Results

![Figure 1. G6PD activity in G6PD deficient whole blood (n=11) and controls whole blood (n=11) expressed as % of 10^6 cells. Data are means±SD. *P<0.05.

Figure 2. PCR-RFLP assay for G6PD variant. PCR-RFLP results in normal control showing a 345 bp band after Xho I digestion (N). G6PD deficiency with 1376 point mutation showing a 324 bp band after Xho I digestion (P).

Figure 3. Base peak chromatograms (BPC) obtained from extracts of control RBCs and G6PD deficient RBCs with or without diamide treatment. Control RBCs (A, B) and G6PD deficient RBCs (C, D) were untreated (A, C) or treated (B, D) with 1 mM diamide for 3 hours and scanned by ESI.

Figure 4. Principal component analysis (PCA) of metabolomes in control and G6PD deficient RBCs. (A) and diamide treatment (B). PCA score plot was shown (control RBCs, red color; G6PD deficient RBCs, cyan color; control RBCs treated with diamide, pikey color; G6PD deficient RBCs treated with diamide, blue color). Control (n = 5) and G6PD deficient RBCs (n = 5) were treated with 1 mM in time course (0 min, square shape; 30 min, circle shape; 60 min, rhombus shape; 120 min, triangle shape; 180 min, rectangular shape). Features were acquired in ESI positive ion mode.

Figure 5. Venn diagram shows the number of metabolites that significantly change (p < 0.05) the level in control and G6PD deficient RBCs upon mock (yellow/pink/diamide treatment (green) at 60 min (A), 120 min (B) and 180 min (C), (D) Heat map show the change in abundance of 220 metabolites that varied in control and G6PD deficient RBCs upon diamide treatment at 60 min 120 min and 180 min.

Figure 7. Purine metabolism in G6PD deficient RBCs and control RBCs upon diamide treatment. LC-MS based approach were used to quantify related abundance of purine metabolism in control (N) and G6PD deficient RBCs. (P) that treated with diamide (control RBCs treated with diamide, NT; G6PD deficient RBCs treated with diamide, PT). Data shown as percentage compared to basal condition of control RBCs at 0 min.

Figure 8. Modification of protein in RBC membrane upon diamide treatment. Lanes 1.6: 0 min; Lanes 2.7: 30 min; Lanes 3.8: 60 min; Lanes 4.9: 120 min; Lanes 5.10: 180 min; Lanes 11.12 were positive control. SDS-PAGE analysis revealed that basal condition (A) treated with diamide (B) induced the appearance of high-molecular weight protein aggregates.

Figure 9. Deformability in normal RBC (A) and G6PD deficient RBC (B). Both groups treated with different dose of diamide (including 0.75 mM green color, 1 mM (green color), 2.5 mM (orange color).

Summary

The level of metabolites increased in G6PD deficient RBCs upon diamide treatment show as red color, the level of metabolites decreased in G6PD deficient RBCs upon diamide treatment show as blue color. CyC: cystine, 2AB: 2-aminobutyrate, g-glu: gamma-glutamylcysteine, g-glu2AB: gamma-glutamyl-2-aminobutyric acid, Ino: inosine, Hyp: hypoxanthine, Xan: xanthine, Gua: guanine, Ado: adenosine, SAM: s-adenosylmethione, SAH: s-adenosylhomocysteine.