Proteomic analysis of glutathione S-transferase isoforms in mouse liver mitochondria

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Abstract

AIM: To survey glutathione (GSH) S-transferase (GST) isoforms in mitochondria and to reveal the isoforms’ biological significance in diabetic mice.

METHODS: The presence of GSTs in mouse liver mitochondria was systematically screened by two proteomic approaches, namely, GSH affinity chromatography/two dimensional electrophoresis (2DE/MALDI TOF/TOF MS) and SDS-PAGE/LC ESI MS/MS. The proteomic results were further confirmed by Western blotting using monoclonal antibodies against GSTs. To evaluate the liver mitochondrial GSTs quantitatively, calibration curves were generated by the loading amounts of individual recombinant GST protein vs the relative intensities elicited from the Western blotting. An extensive comparison of the liver mitochondrial GSTs was conducted between normal and db/db diabetic mice.

RESULTS: Using GSH affinity/2DE/MALDI TOF/TOF MS, three GSTs, namely, alpha3, mu1 and pi1, were identified; whereas five GSTs, alpha3, mu1, pi1, kappa1 and zeta1, were detected in mouse liver mitochondria using SDS-PAGE/LC ESI MS/MS, of these GSTs, GST kappa1 was reported as a specific mitochondrial GST. The $R^2$ values of regression ranged between values of about 0.86 and 0.98, which were acceptable for the quantification. Based on the measurement of the GST abundances in liver mitochondria of normal and diabetic mice, the four GSTs, alpha3, kappa1, mu1 and zeta1, were found to be almost comparable between the two sets of animals, whereas, lower GST pi1 was detected in the diabetic mice compared with normal ones, the signal of Western blotting in control and db/db diabetic mice liver mitochondria is $134.61 \pm 53.84$ vs $99.74 \pm 46.2$, with $P < 0.05$.

CONCLUSION: Our results indicate that GSTs exist widely in mitochondria and its abundances of mitochondrial GSTs might be tissue-dependent and disease-related.

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Key words: Glutathione S-transferase; Mitochondria; Liver; Proteomics; Diabetes

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Student's $t$ test was adopted for the estimation of regression and significant difference.
INTRODUCTION

The glutathione (GSH) S-transferase (GST, EC2.5.1.18) superfamily contains eight subclasses classified by their properties of sequence homology, immunology, substrate specificity and isoelectric point. GSTs catalyze the reactions between reduced GSH and unsaturated aldehydes, quinines, and many other substrates, especially under conditions of oxidative stress\[^{[1-3]}\]. These enzymes are involved in many physiological functions, such as the reduction of free radical damage, detoxification, metabolism, regulation of cell signaling and nitric oxide storage\[^{[2-7]}\]. The abundance of GSTs is closely related with the disease status of the organism\[^{[8,12]}\]. For instance, regarding a cancer biomarker, GST pi was found in higher abundance in several cancer cells and tissues and was believed to be involved in drug resistance\[^{[13-15]}\]. GST alpha has main functions in detoxification in the liver, and when hepatocytes are damaged, GST alpha enters the bloodstream. Therefore, GST alpha in blood and urine is an ideal marker indicating hepatocellular impairment\[^{[16]}\]. The accurate measurement of GSTs in tissues or body fluids is urgently required in biomedicine.

GSTs have been identified mainly in the cytoplasm, but they have also recently been detected in organelles, including the microsome, nucleus, mitochondria and peroxisomes\[^{[17-20]}\]. Mitochondria are the primary intracellular sites of oxygen consumption and reactive oxygen species (ROS) generation. GST kappa1 was identified in mitochondria and peroxisomes but is absent in the cytoplasm, and GST kappa was reported to participate in energetic and lipid metabolism in the mitochondria\[^{[21]}\]. In contrast to the cytoplasmic GSTs, the physiological functions of the mitochondrial GSTs have yet to be explored. Generally, it is accepted that GSTs are able to protect mitochondria from dysfunctions of catalase and superoxide dismutase by maintaining the redox balance\[^{[22-24]}\]. A high abundance of GST pi protected the decrease of the mitochondrial membrane potential induced by rotenone. Raza group observed that under oxidative stress, GST alpha was translocated from the cytoplasm into the mitochondria, but its functions in the mitochondria were not elucidated\[^{[25]}\]. To understand the functions of the mitochondrial GSTs fully, we must first define how many GST isoforms exist in the mitochondria. Although there were several reports regarding the distribution of GSTs in different tissue mitochondria, a systematic investigation in this field has not been undertaken.

Recently, proteomic approaches have provided a good opportunity to survey the members of a protein family, as they usually share similar properties, such as immunofinity, ligand binding sites, homology sequences and catalytic substrates. Using chromatography or electrophoresis based on these properties, protein family members could be separated from one another in a mixture. Moreover, mass spectrometry is able to identify the separated proteins. Such a proteomics strategy has been widely used in exploring protein isoforms\[^{[25,27]}\]. In this study, we propose a proteomic strategy to define the GSTs in mouse tissue mitochondria. With combined separation based on size exclusion and affinity chromatography, we enriched the mitochondrial GSTs from a mitochondria preparation of mouse liver. Using MALDI TOF/TOF MS or ESI MS/MS, five GSTs were identified in the mitochondria. The presence of GSTs was further verified by Western blot using monoclonal antibodies. We constructed calibration curves of the GST quantification and employed a quantitative assay of the immunoblots to estimate the different abundances of the mitochondrial GSTs between normal and diabetic mice. For the first time, we found that GSTs are widely distributed in the mitochondria of many tissues and that mitochondrial GST pi1 is sensitive to the development of diabetes.

MATERIALS AND METHODS

Preparation of mitochondria from mouse tissue

The C57BLKS/J db/db and control mice were provided by Peking University Diabetes Center. The heart, kidney and liver tissues of the mice were minced and homogenized in buffer (25 mmol sucrose, 0.5% protease inhibitor cocktail and 10 mmol HEPES, pH 7.4). The crude mitochondria were prepared by differential centrifugation at 1000 × g for 30 min and at 10 000 × g for 20 min at 4 ℃. The purified mitochondria were extracted from a Nycodenz gradient at the interface of 25%-30% Nycodenz solution after centrifugation at 52 000 × g for 90 min. The purity and integrity of the mitochondria were determined by Western blotting and transmission electron microscopy (TEM). Mitochondrial proteins were extracted using lysis buffer [7 mol/L urea, 2 mol/L thiourea, 4% CHAPS, 40 mmol/L Tris-HCl (pH 7.4) and protease inhibitor cocktail].

The animal experiments described in this article were approved by the Animal Care and Welfare Committee at the Beijing Institute of Genomics, Chinese Academy of Sciences.

GST-affinity chromatography

We purified the GSTs using GST-affinity chromatography with GST-Sepharose 4B (Amersham Biosciences, United states). The GST-Sepharose 4B was equilibrated with binding buffer [150 mmol/L NaCl, 50 mmol/L Tris-HCl (pH 8.0), 1 mmol/L ethylene glycol tetracetic acid, and 0.1% Triton X 100]. The mitochondria were resuspended in 500 µL binding buffer and were sonicated. After centrifugation, the supernatant was mixed with the equilibrated resin and centrifuged for 30 min 3000 r/min at 4 ℃. The affinity resin was washed 3 times with binding buffer, and the proteins were eluted from the resin using 30 mmol/L reduced GSH. A sample of the elution products was retained for two-dimensional electrophoresis (2-DE) separation.

2-DE

The first dimension separation was conducted using an
Ettan IPGphor IEF system with 7 cm (pH 6-11) IPG strips at 20 °C. The proteins isolated by GSH-affinity chromatography were loaded onto strips, and the strips were rehydrated without voltage for 4 h and with 50 V for 8 h. The isoelectric focusing was programmed for 1 h at 500, 1000 and 4000 V, respectively, and was subsequently focused at 4000 V up to a total of 30 kVh. The focused strips were equilibrated in buffer with 6 mol/L urea, 50 mmol/L Tris-HCl, 30% glycerol, 2% SDS and trace bromophenol blue and were subsequently reduced by dithiothreitol and alkylated by iodoacetamide. The treated strips were inserted into a 15% SDS-PAGE gel running in 2.5 W (each gel) for 30 min and 15 W (each gel) thereafter until the bromophenol blue dye reached the bottom of the gels. The gels were stained by silver staining.

**Mass spectrometry for protein identification**

The proteins were identified by two mass spectrometry methods: MALDI TOF/TOF and LC ESI MS/MS.

The proteins that were separated by GSH-affinity chromatography and 2D gel electrophoresis were excised and in-gel digested with trypsin overnight and identified by MALDI TOF/TOF MS. Briefly, the tryptic digests were co-crystallized with a matrix of α-cyano-4-hydroxycinnamic acid spotted onto the AnchorChip and desalted by 0.1% trifluoroacetic acid. The AnchorChip was analyzed using an Ultraflex TOF/TOF MS mass spectrometer (Bruker Dalton, Bremen, Germany) for protein identification. Positively charged ions were analyzed in the reflector mode. Typically, 100 shots were cumulated per spectrum in the MS mode and 400 shots in the MS/MS mode. The mass spectra and tandem mass spectra obtained were processed using the FlexAnalysis 2.2 and BioTools 2.2 software tools. The protein identification was performed using the Mascot software (http://www.matrixscience.com), and the NCBIInr database was searched using mouse as the taxonomy. The following parameters were used for the database searches: one incomplete cleavage, alkylation of cysteine by carbamidomethylation, oxidation of methionine, and pyro-Glu formation of the N-terminal Gln.

The 20-30 kDa proteins separated by SDS-PAGE were a mixture of many proteins, and the proteins were examined by LC ESI MS/MS after the in-gel trypsin digestion. Briefly, after capillary reversed-phase high-performance liquid chromatography, the separated peptides were analyzed using an ion-trap mass spectrometer LCQ DecaXP ion-trap mass spectrometer (Thermo Finnigan, Bremen, Germany) with 3.2 kV of spray voltage and 150 °C at the heated desolvation capillary. A mass-to-charge (m/z) range from 400 to 2000 was scanned over 1.2 s, and the ions were detected with a high energy conversion dynode detector. The MS/MS data were converted into DTA-format files, which were further searched for proteins with Sequest software. All of the accepted results had a deltaCn of 0.1 or greater. Furthermore, a singly charged peptide must be tryptic, and the cross-correlation score (Xcorr) had to be more than 1.9. The tryptic peptides with a charge state of + 2 must have a Xcorr of more than 2.2. Triply charged tryptic peptides were accepted if the Xcorr was ≥ 3.0.

**Generation of monoclonal antibodies**

To generate the specific antibodies against these five GST isoforms, we cloned these five GST genes and expressed the recombinant protein by inserting these genes into the prokaryotic expression vector, named pET-28a. The recombinant proteins expressed from the pET-GST plasmids were 6× His-tagged. After expression in *E. coli* BL21 (DE3), the proteins were purified using Ni-NTA agarose resin (Qiagen, United States) and metal chelate chromatography.

Six-weeks-old female BALB/c mice were immunized subcutaneously with the recombinant protein in Freund's complete adjuvant. After booster injections, the mice with positive serum immunogenicity against the recombinant protein were used for the monoclonal fusion experiments. The mice’s spleens were excised, and single-spleen cell suspensions were fused with Sp2/0 myeloma cells. After several days of fusion, the hybridomas were picked and cultured in complete medium supplemented with 1% HT. Ascitic fluids were collected from the mice after an intraperitoneal injection of the hybridomas, and the antibodies were purified by protein A/G-Sepharose affinity chromatography. The protein concentrations of the purified antibodies were determined using the Bradford method, and the antibodies were diluted to 1 mg/mL and stored in 50% glycerin at -20 °C.

**Western blotting**

Proteins were separated by 15% SDS-PAGE and were electro-transferred onto polyvinylidene fluoride (PVDF) membranes. The PVDF membranes were blocked with 5% non-fat milk dissolved in Tris-buffered saline with 0.05% Tween-20 (TTBS) at 37 °C for 90 min. The membranes were incubated with the primary antibodies at a dilution of 1:5000 in blocking reagent at room temperature for 2 h. The antibodies against the GSTs were generated by our laboratory, and the anti-ATP synthase β antibody was purchased from BD Biosciences. The membranes were incubated in goat anti-mouse/rabbit IgG conjugated with horseradish peroxidase at a 1:3000 dilution at room temperature for 1 h. The membranes were developed using the Super ECL Plus Detection Reagent kit, and the images were captured using ImageQuant ECL (GE Healthcare, United Kingdom).

**Statistical analysis**

To quantify the GSTs, calibration curves were constructed using the protein concentrations and immunosignal obtained from the Western blotting. To analyze the quantities of GSTs in the diabetes mouse model statistically, the relative intensity of the Western blotting signals in the samples was quantified using ImageQuant TL software. The three pairs of samples from the control and db/db mice were then randomly paired, and the relative abundance ratios of the five GST proteins were...
statistically analyzed and were normalized using the levels detected in normal mice based on two parallel experiments. All of the values are expressed as the mean ± SD, and the statistical significance was set to a $P < 0.05$.

**RESULTS**

**Screening of GST isoforms in liver mitochondria**

Considering that the purity of the mitochondria is a key element in studying mitochondrial components, Nycodenz gradient centrifugation was employed for the preparation of the mouse liver mitochondria. The mitochondrial integrity and purity were examined by Western blot and TEM. Our data as shown in Figure 1 indicated that the purity and integrity of the mitochondria were satisfactory for further analysis.

The mitochondrial lysates were loaded onto GSH affinity columns, and the fractions eluted by GSH were collected and subjected to 2DE using pH 6-11 strips and 15% SDS-PAGE. After silver staining, a string spots of approximately 28 kDa and pI 7-10 appeared on the gels (Figure 2), whereas no spots were detected in other parts of the gel, indicating that the affinity enrichment was effective to remove non-GSH-binding proteins. The silver-stained spots were excised and digested with trypsin followed by protein identification using MALDI TOF/TOF MS. In total, three GSTs, GST alpha3, GST mu1 and GST pi1, were found in the liver mitochondria. Because all of the GSTs have similar MWs (about 28 kDa), we attempted to separate the mitochondrial GSTs from the other mitochondrial proteins through size-exclusion methods. The mitochondrial lysates were loaded onto a 15% SDS-PAGE gel, and the portion corresponding to 20-30 kDa was sliced into 19 fractions. These fractions were digested with trypsin, and the digested fractions were further analyzed using LC ESI MS/MS. All of the unique peptide sequences of GSTs are listed in Table 1, indicating five GSTs: GST pi1, GST alpha3, GST mu1, GST kappa1 and GST zeta1. All of the GSTs identified by GSH/2DE/MALDI TOF/TOF MS are included in the list of the identified GSTs by SDS-PAGE/LC ESI MS/MS; therefore, the SDS-PAGE/LC ESI MS/MS approach exhibits a better sensitivity for the detection of GSTs.

The existence of GSTs in the liver mitochondria was further confirmed by Western blotting using monoclonal antibodies against the individual GSTs (the specificity of detection is described below). As depicted in Figure 3, the five GST isoforms detected by the proteomic methods showed positive immunoreactivity; this evidence strongly supports the conclusion reached with the proteomic analysis. Furthermore, we prepared the tissue mitochondria from mouse kidney and heart and conducted Western blotting to evaluate the abundance of the GSTs in the mitochondria from different tissues. The left panel in Figure 3 reveals the wide distribution of GSTs in mitochondria.
the mitochondria from the three tissues with relatively different abundances. As a specific GST only located in mitochondria, GST kappa1 exists in all three tissues with similar abundances. GST pi1 is clearly detected in the mitochondria of all three of the tissues, whereas GST pI1 in the liver mitochondria is in the highest abundance. The three GSTs, GST alpha1, GST mu1, and GST zeta1, are clearly observed in the liver and kidney mitochondria, whereas they are almost undetected in the heart. Furthermore, we examined the cytoplasmic GSTs and the mitochondrial GSTs in the mouse liver using Western blotting. The data illustrated in the right panel in Figure 3 indicate that GST kappa1 is the only isoform undetectable in the cytoplasm, whereas the other GST isoforms are readily detected in the two subcellular fractions. When equal amounts of proteins, either cytoplasmic or mitochondrial, are loaded onto an SDS-PAGE gel, the analysis of the relative intensities demonstrate that the abundances of GSTs in the cytoplasm are commonly higher than those in the mitochondria.

**Quantitative analysis of the mitochondrial GST using Western blotting**

The five GSTs in liver mitochondria were first discovered by proteomic approach. As a routine assay in laboratory, mass spectrometry is not a common instrument and easy in use. Western blotting is still a widely acceptable and enough sensitive approach to detect proteins. We therefore developed a quantitative assay for the mitochondria GSTs based on immuno-blot. It is well known that specificity and sensitivity of antibody are a key factor for a successful Western blotting. To have the qualified antibodies against GSTs, we generated a set of monoclonal antibodies of GSTs, and performed a strict screening. A qualified antibody should match to criteria: (1) It can recognize 2 ng of the correspondent recombinant protein; and (2) It cannot pick up any signals to 20 ng of unrelated GSTs. The qualified GST antibodies are illustrated in the upper panel of Figure 4.

Selection of the concentration ranges for the recombinant GSTs is an important consideration for quantitative calibration. In our routine experiments, the maximum loading of mitochondrial proteins are approximately 20 µg; therefore, we loaded 20 µg of proteins and different amounts of recombinant GSTs on a relatively large scale onto the same gels and performed the Western blotting analysis. After determining the band intensities, we were able to estimate the proper amounts of the recombinant GSTs for the generation of calibration curves. As shown in Figure 4, five calibration curves are generated corresponding to the multiple assays of our Western blotting. However, Western blotting has a disadvantage in quantitative measurements because the reproducibility of the band intensities is relatively poor. The data shown in Figure 4 is in agreement with the observation, although some spots indeed display a large deviation. However, the regression calculation revealed that the values of \( R^2 \) ranged from 0.85-0.98, which are generally accepted in quantitative measurements using Western blot. We further estimated the possible dynamics of the GST abundances in the liver mitochondria. With the exception of GST pi1, measuring approximately 50 ng to 300 ng, the other four GSTs were between 10 ng to 40 ng, indicating that GST pi1 has the highest abundance in liver mitochondria. According to the regression curves in Figure 4, the mitochondrial GST abundances were quantitatively estimated as follows: 134.61 + 53.84, 91.33 + 29.25, 43.83 + 23.33, 29.25 + 20.29, 27.28 + 0.27, 15.84 + 0.16 ng per µg mitochondrial protein for GST pi1, mu1, alpha3, kappa1, and zeta1, respectively.

**Comparison of the GSTs’ abundances in liver mitochondria between normal and diabetes mice**

The db/db mouse is a well-accepted type II diabetes model, and previous studies have shown that free radicals were increased in most of the tissues in this model. After carefully examining the mice’s blood glucose and body weight to ensure diabetes development, we collected the livers from three normal and three db/db mice; the prepared mitochondria were analyzed by Western blot to quantify the contents of the GSTs. Figure 5A shows such a typical Western blotting, suggesting that the immunoreactivity of GST pi1 is significantly attenuated in the mitochondria of the diabetic mice, the signal of Western

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**Table 1 The unique peptides of glutathione S-transferases identified by LC ESI MS/MS**

| GST   | Peptide sequence       | MH+   | Location | XC   | DeltaCn | Ions |
|-------|------------------------|-------|----------|------|---------|------|
| Mu1   | MLLEYTDSYDEK           | 1593.699 | 19-31   | 4.062 | 0.47    | 20 [24] |
|       | ADIVENQMDTR            | 1390.664 | 97-108  | 4.1055 | 0.5208  | 17 [22] |
|       | MQILMLCNYDFPEK         | 1801.811 | 109-122 | 4.8593 | 0.5987  | 20 [26] |
| Zeta1 | GIDYEIEPINLK           | 1486.852 | 28-40   | 3.0229 | 0.3943  | 15 [24] |
|       | VITSSFNALEK            | 1178.642 | 134-144  | 2.6217 | 0.4564  | 16 [20] |
| Kappa1| FLTTSMSQPEMMEK         | 1782.866 | 102-116 | 4.944  | 0.5723  | 22 [36] |
|       | AGMSTAQAQHFKLEK        | 1518.737 | 145-158 | 2.4224 | 0.2908  | 13 [16] |
| Alpha3| SDGSIMQOQPVMEIDGMK     | 2111.982 | 46-64   | 3.9127 | 0.5378  | 21 [28] |
|       | ALNYIAK                | 992.578  | 70-78   | 3.0611 | 0.3416  | 17 [26] |
|       | YFPAFEK                | 901.446  | 132-138  | 2.5832 | 0.3767  | 8 [12]  |
| Pi1   | EAAQMDMVDNGVEDLR       | 1792.785 | 86-101  | 3.9673 | 0.5391  | 22 [30] |
|       | YVTLIYNTNGK            | 1577.785 | 104-116  | 3.6743 | 0.4268  | 18 [24] |

MH+ stands for the mass of ions detected by LC ESI MS/MS. XC stands for the cross-correlation score. GST: Glutathione S-transferase.
blotting in control and db/db diabetic mice liver mitochondria is 134.61 ± 53.84 vs 99.74 ± 46.2, with $P < 0.05$, whereas the other four GST isoforms are nearly comparable between the normal and diabetic mice. Statistically, we performed pairwise comparisons and estimations of the significant differences based on multiple Western blotting. The comparison data depicted in Figure 5B support the conclusion drawn from Figure 5A that the abundance of mitochondrial GST pi1 in diabetic mice is lower than in normal mice, whereas the other mitochondrial GSTs did not exhibit significant changes.

We screened the response of the GSTs to diabetes in the db/db liver mitochondria. As depicted in Figure 5A, which shows representative images of mitochondrial tissue from one pair of mice (control vs db/db), GST pi1 showed a decrease in the db/db mouse when we compared three pairs of mice. In contrast, similar expression levels of GST alpha3, mu1, and zeta1 were observed in the liver mitochondria of the db/db mice and control mice. The statistical analysis indicated in Figure 5B supported these observations.

**DISCUSSION**

GSTs have been detected as a group of oxidative stress proteins in mitochondria, and they were characterized as associated with the maintenance of mitochondria functions \(^{[22,28]}\). For example, alpha-class isozymes of GST translocated into the mitochondria under oxidative stress, and the isoforms showed glutathione peroxidase activity toward phospholipid hydroperoxide in the rat liver cytosol. Chemicals that generate reactive oxygen species, such as rotenone and antimycin A, reduced the cell viability and mitochondrial membrane potential, and the overexpression of GST pi diminished these changes.

In general, GSTs were determined by their enzymatic activities and immunological features; however, these methods had limitations in evaluating the presence of GST. The substrate specificity of purified rat liver GSTs has been investigated by a series of gamma-glutamyl-modified GSH analogues, and GST had a different ability to conjugate with the substrates. Furthermore, a product of the purification using GSH could not provide the status of GST \textit{in vivo}. Therefore, the uncertainty of the type of GSTs localized in the mitochondria and the antibody specificity led to the uncertainty of an increase or decrease of GSTs under oxidative stress or in a pathological condition.

In this study, we have taken multiple approaches to identify the GSTs in mouse liver mitochondria. The mitochondrial proteins were separated by SDS-PAGE, and the 20-30 kDa proteins were analyzed by liquid chromatography and subsequently identified by ESI MS/MS. Five GST isoforms were detected in the liver mitochondria. Our strategy has two advantages: more proteins could be resolved regardless of the solubility by SDS-
Normal mice and found that the GST response to oxidative stress varies: only GST pi1 is decreased in the db/db mouse compared to normal mice, and the levels of the other four isoforms did not change. This result indicated that a decrease of GST pi1 might accelerate the pathological progress of diabetes.

**Figure 5** Comparison of mitochondrial glutathione S-transferase abundances between normal and diabetic mice by Western blotting. A: A typical image of a Western blotting comparing a pair of mouse liver mitochondria from normal and diabetic mice. Approximately 20 µg mitochondrial proteins were loaded in each lane. Anti-ATP synthase β acted as a control to normalize the loading; B: The statistical comparison of the relative ratio of mitochondrial glutathione S-transferases (GSTs) between normal and diabetic mouse liver when normalized by the GSTs in normal mice (n = 3).

**COMMENTS**

**Background**

The glutathione (GSH) S-transferase (GST) involve in many physiological functions. They are located mainly in the cytoplasm, but they have also been detected in suborganelles. Because mitochondria are the primary intracellular sites of oxygen consumption and reactive oxygen species generation, the mitochondrial GSTs abundances may relate with disease status. The goal was to survey GST isoforms in mitochondria and to reveal their biological significance in diabetic mice.

**Research frontiers**

It is reported that GSTs are able to protect mitochondria from oxidation by maintaining the redox balance, but their functions in the mitochondria were not elucidated. To fully understand the functions of the mitochondrial GSTs, new method to monitor the mitochondrial GSTs is urgently required.

**Innovations and breakthroughs**

The mitochondrial GSTs are measured by the combined quantitative proteomic strategies based on mass spectrum and antibodies. The authors discovered that GSTs are widely distributed in tissue mitochondria and their responses to diabetes physiology. It is noted the mitochondrial GST pi1 is sensitive to diabetic development.

**Applications**

This research showed how the proteomics produces meaningful information and offered a relatively easy and simple workflow to detect GSTs in liver mitochondria and to reveal their biological significance of normal and diabetes mouse. At the same time, the application of this strategy provides an alternative tool to analyze how isoforms of protein family response to disease in complex biological systems.

**Terminology**

Proteomics is the protein map of a biological system and involves the systematic study of proteins in order to provide a comprehensive view of the function and regulation of proteins.

**Peer review**

This paper provides solid data on systematic analysis of GST isoforms in mouse liver mitochondria.
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