Oxidative Modification of Hepatic Mitochondria Protein Thiols: Effect of Chronic Alcohol Consumption

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Running title – Alcohol and oxidized mitochondrial protein thiols

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ABSTRACT

Redox modification of mitochondrial proteins is thought to play a key role in regulating cellular function although direct evidence to support this hypothesis is limited. Using an in vivo model of mitochondrial redox stress, ethanol hepatotoxicity, the modification of mitochondrial protein thiols was examined using a proteomics approach. Specific labeling of reduced thiols in the mitochondrion from the livers of control and ethanol fed rats was achieved by using the thiol reactive compound (4-iodobutyl) triphenylphosphonium (IBTP). This molecule selectively accumulates in the organelle and can be used to identify thiol containing proteins. Mitochondrial proteins that have been modified are identified by decreased labeling with IBTP using two-dimensional SDS-PAGE followed by immunoblotting with an antibody directed against the triphenylphosphonium moiety of the IBTP molecule. Analyses of these data showed a significant decrease in IBTP labeling of thiols present in specific mitochondria matrix proteins from ethanol–fed rats as compared to their corresponding controls. These proteins were identified as the low $K_m$ aldehyde dehydrogenase and glucose regulated protein 78 (GRP78). The decrease in IBTP labeling in aldehyde dehydrogenase was accompanied by a decrease in specific activity of the enzyme. These data demonstrate that mitochondrial protein thiol modification is associated with chronic alcohol intake and might contribute to the pathophysiology associated with hepatic injury. Taken together, we have developed a protocol to chemically tag and select, thiol modified proteins, which will greatly enhance efforts to establish post-translational redox
modification of mitochondrial protein in in vivo models of oxidative or nitrosative stress.

**Key words** – Mitochondrial thiol proteins, cysteine, reactive oxygen species and reactive nitrogen species
INTRODUCTION

Mitochondria have received considerable attention as a principal source and target of reactive oxygen and nitrogen species (ROS/RNS) with mitochondrial damage and dysfunction observed in a number of pathologies including alcoholic liver disease, myocardial preconditioning, and ischemia-reperfusion injury (11, 13, 29, 30). Mitochondria also play an important role in cellular redox signaling via several mechanisms, although few specific mitochondrial targets have been identified. Potential alterations to protein thiols that can alter function include the formation of mixed disulfides or internal disulfides from vicinal dithiols, S-nitrosation of thiol groups, and the formation of higher oxidation states such as sulfenic, sulfinic, or sulfonic acids (24). Even though increased levels of oxidized protein thiols are commonly used as an indicator of oxidative damage in mitochondria, the identity of specific mitochondrial proteins modified during oxidative stress in vivo are largely unknown.

Recently, a method using a novel lipophilic compound (4-iodobutyl) triphenyl phosphonium (IBTP) was established, which selectively labels reduced thiol groups within mitochondrial proteins (15, 25). This lipophilic cation is concentrated in the organelle due to the large membrane potential across the inner mitochondrial membrane. IBTP was shown to co-localize with mitochondrial enzymes in human fibroblasts with essentially uniform labeling of all mitochondria (15). Once IBTP is taken up into the mitochondrion, the thiolate groups present within proteins displace the iodo group of IBTP to form a stable phosphonium thioether. Selective labeling of mitochondrial proteins is dependent
upon the membrane potential since uncouplers essentially remove all IBTP adducts in both cells and isolated mitochondria. These IBTP-labeled proteins can easily be identified by immunoblotting with an antibody raised against the triphenylphosphonium group. Thus, thiols present within proteins that have been oxidized or modified as a consequence of oxidants can be identified by decreased labeling with IBTP using gel electrophoresis and immunoblotting with the anti-triphenylphosphonium antibody (anti-TPP) (15).

It was predicted that utilization of this technique in combination with a mitochondrial proteomics approach would enable the identification of specific mitochondrial proteins which are modified in an in vivo model of oxidative or nitrosative stress. For these studies, we chose a model of chronic alcohol consumption that is known to induce mitochondrial production of ROS/RNS, oxidation of mitochondrial proteins, and mitochondrial dysfunction (3, 4, 8, 30). Thus, a protocol was developed in which we were able to identify specific mitochondrial proteins that had altered thiol redox status as a consequence of chronic alcohol exposure. Detection of these post-translational modifications demonstrates at the molecular level a mechanism by which chronic alcohol consumption might negatively affect mitochondrial energy metabolism and contribute to alcohol-induced liver toxicity. Furthermore, the results of these studies demonstrate that this approach can be extended to a wide variety of other pathophysiological conditions where oxidative and nitrosative stress has been implicated in the etiology.
MATERIALS AND METHODS

Materials - Lieber-DeCarli liquid diets were purchased from Bio-Serv (Frenchtown, NJ). Carrier ampholines for isoelectric focusing gels (pH 3-10, 4-6, 5-8) and donkey anti-rabbit IgG HRP conjugate were purchased from Amersham Pharmacia Biotech (Piscataway, NJ). The matrix α-cyano-4-hydroxycinnamic acid was purchased from Aldrich (St. Louis, MO). IBTP and the rabbit polyclonal antibody against thiobutyltriphenylphosphonium were prepared as described in (15). Anti-rat liver mitochondrial aldehyde dehydrogenase IgG was kindly provided by Dr. Henry Weiner, Purdue University, West Lafayette, IN.

Animals and diets - Male Sprague-Dawley rats (200g) were individually housed in suspended cages and maintained under a 12 hr light/dark cycle for the entire duration of the feeding protocol. Nutritionally adequate ethanol and control liquid diets were formulated and prepared according to Lieber and DeCarli (14). The ethanol diet provides 36% of the total daily caloric intake as ethanol, 11% as carbohydrate, 18% as protein, and 35% as fat. Weight-matched control rats were pair-fed and received identical diets except that ethanol calories were substituted isocalorically by dextrin maltose. Animals were maintained on the diets for at least 31 days as described by (2, 4).

Isolation of rat liver mitochondria and labeling with IBTP - Coupled liver mitochondria were prepared by differential centrifugation of liver homogenates (23, 26) using ice-cold mitochondria isolation medium containing 0.25 M sucrose,
1 mM EDTA, and 5 mM Tris-HCl, pH 7.5. Protease inhibitors were added to the isolation buffer to prevent protein degradation (2, 4). The total mitochondria protein from control animals was 224 +/- 20 mg and from ethanol treated animals 257 +/- 10 mg (mean +/- SEM, n=8, p=0.19). Respiratory control ratios for mitochondria isolated from control animals was 4.9 +/- 0.3 and for ethanol-fed animals 3.5 +/- 0.2 (mean +/- SEM, n=8, p=0.0004).

For IBTP labeling of reduced mitochondrial protein thiols, mitochondria (1.0 mg/mL) were incubated at 37°C in respiration buffer containing 120 mM KCl, 5 mM KH2PO4, 1 mM EGTA, 25 mM sucrose, 5 mM MgCl2, and 3 mM HEPES, pH 7.4 and respiration was initiated by the addition of succinate and ADP. After 3 min, 5 µM IBTP was added to mitochondria and the reaction was allowed to proceed for 10 min. The reaction was stopped by the addition of iodoacetic acid. Control experiments were also performed with labeling in the presence of FCCP. Incubation of mitochondria with this concentration of IBTP has no effect on mitochondrial respiration and does not deplete mitochondrial glutathione (15). Mitochondrial suspensions were centrifuged at 12,500 rpm, the supernatant was discarded, and pellets were stored at -80°C until used for gel electrophoresis.

**Electrophoresis and immunoblotting** - For one-dimensional (1D) SDS-PAGE mitochondrial samples from control and ethanol-fed rats were separated on 10-18% gradient gels, transferred to nitrocellulose membranes and probed with a 1:10,000 dilution of anti-TPP followed by incubation with 1:3,000 dilution of donkey anti-rabbit HRP conjugated secondary antibody. For two-dimensional
(2D) gel electrophoresis, mitochondria were suspended in tube gel sample buffer (9.5 M urea, 2.0% CHAPS, 1.0% DTT, and 0.8% (v/v) of each of pH 3-10, 5-8, and 4-6 ampholines). After 1 hr incubation to fully solubilize mitochondria membranes, 50 µg of protein was loaded onto isoelectric focusing gels (pH gradient 4-8.5) and focused overnight for 16 hr at 400V, followed by 1hr at 800V to sharpen bands. Isoelectric focusing gels were equilibrated in 62.5 mM Tris-HCl, 3.0% SDS, 10% glycerol, 0.005% bromophenol blue, and 1.0% DTT before being loaded onto 8-20% gradient gels for SDS-PAGE. For each control and ethanol pair of samples, gels were run in duplicate with one gel Coomassie blue-stained and the other to be used for immunoblotting with the anti-TPP antiserum. The anti-TPP membranes were incubated with a 1:30,000 dilution of the anti-TPP antiserum for 1 hr followed by incubation with a 1:5,000 dilution of secondary antibody for 1hr. IBTP-labeled protein thiols for both 1D and 2D gels were visualized by ECL detection.

**Image analysis of two-dimensional gels and anti-TPP blots** - For Coomassie blue-stained gels, each control and its matching ethanol pair were stained and destained in an identical manner. Gels were scanned and were used for analysis with PDQuest software (BioRad Hercules, CA). Protein spots were identified using the spot wizard tool and a match set was created containing all gels so that matched protein spots would be given the same identification number. The control gel from one pair was used as the master image for protein spot matching. For each pair of gels, the total protein density in the entire gel was
nearly identical (control, 971,715 +/- 277,153 vs. ethanol 1,045,240 +/- 348,049 relative density units, mean +/- SEM, p=0.43), thus normalization of individual protein spot densities did not change the outcome of the analysis. We did not observe any significant differences among the matched spots on the Coomassie-blue stained gel.

For anti-TTP western blots, three pairs of control and ethanol blots were processed and developed together to facilitate comparison. Blot images were obtained using FluorChem software on an Alphalnntech camera imager (San Leandro, CA) and the resulting TIFF files were analyzed using PDQuest software. Protein spots were identified as described above. In order to correct for possible inter-gel protein loading differences, the density for each protein spot on the immunoblot was normalized to the total protein density in the corresponding Coomassie blue-stained gel. Normalized spot densities from control blots were compared to normalized spot densities from ethanol blots using a one-tailed paired Student’s t-test.

**Identification of IBTP-labeled mitochondrial proteins** - Protein spots immunoreactive for IBTP labeling were cut from gels and processed for identification using matrix-assisted laser desorption ionization/time-of-flight (MALDI-TOF) mass spectrometry essentially as described by Brookes and colleagues (5). Films and images of IBTP-immunoreactive protein spots were aligned with the duplicate stained gels so that the corresponding immunoreactive protein spot could be identified. MALDI-TOF analysis was accomplished using a PE-
Biosystems Voyager Elite instrument (Framingham, MA) equipped with a nitrogen laser (337 nm) and operated using a delayed extraction mode. The peptide masses were entered into “Mascot” (http://www.matrixscience.com) and the NCBI database was searched to identify the proteins.

**Aldehyde dehydrogenase activity and immunoblotting** - Mitochondrial aldehyde dehydrogenase (ALDH) activity was measured by the procedure described previously (16). Mitochondria were pretreated with pyrazole (0.2mM), rotenone (2µM), and allopurinol (20µM) to inhibit alcohol dehydrogenase, NADH dehydrogenase, and xanthine oxidase activities, respectively. The specific activity of ALDH is expressed as nmol NADH produced/min/mg protein and was determined over the linear portion of the reaction. ALDH protein was quantified by immunoblotting with an anti-rat liver ALDH antibody (32).

**Statistical analysis** - Data are reported as means +/- SEM for sample sizes of 3-8 and statistical evaluations between samples means were made by paired Student’s t-test. The minimum level of significance was set at p ≤ 0.05.

**RESULTS**

For these studies, liver mitochondria were isolated from control and ethanol-fed animals and characterized by measurement of coupled respiration and the activity of specific respiratory complexes (23, 26). As reported previously (23, 30) a decrease in both succinate and glutamate/malate respiration was observed
in mitochondria from ethanol-fed animals as compared to controls (results not shown). The specific activity of citrate synthase was 0.179 +/- 0.01 µmol/min/mg protein in control mitochondria, which is similar to reported values and was no different for the ethanol treated animals. This result indicates that the degree of mitochondrial purity was not affected by ethanol treatment. This preparation does contain some minor contamination from other non-mitochondrial membrane components but is consistently functionally viable for the incubation periods required for functional analysis and proteomics. In the first series of experiments mitochondria were treated with IBTP to label exposed thiols while respiring in the presence of succinate and ADP. By labeling respiring mitochondria we were able to identify those reactive thiols that are exposed within mitochondria during oxidative phosphorylation. After establishing electron transport, IBTP (5 µM) was added and the samples were incubated for a further 10 min after which iodoacetic acid (1mM) was added to block mitochondrial thiols. In control experiments FCCP (1µM) or iodoacetic acid (1 mM) was added prior to IBTP.

In the first series of experiments the extent of IBTP labeling was assessed using a low-resolution separation of mitochondrial proteins by 1D SDS-PAGE (Figure 1A) and immunoblotting against IBTP (Figure 1B). These experiments are important to determine whether the extent of IBTP labeling in mitochondria from control and ethanol-fed rats was similar. It is clear that there is no significant difference in the density of the five major bands resolved on the 1D gel with respect to either the protein or IBTP immunoreactive labeling (Figure 1A-C). As expected, labeling with IBTP was prevented by abolishing the mitochondrial
membrane potential with the uncoupler FCCP or by pretreating the mitochondria with the thiol-alkylating agent iodoacetic acid before the addition of IBTP (Figure 1B). Taken together, these data demonstrate that the labeling of mitochondrial proteins by IBTP is dependent on the presence of the mitochondrial membrane potential and that the reaction of IBTP is specific to thiols.

In the low-resolution 1D SDS-PAGE gels the labeling of individual proteins is likely to be masked by the co-migration of proteins with very similar molecular weights. In the next series of experiments the high-resolution separation of matrix mitochondrial proteins was performed using separation in the 1D by IEF followed by 2D SDS-PAGE. The inner membrane proteins are under-represented by this technique since they precipitate in the first dimension of the Isoelectric focusing gel. However, we chose to concentrate on the matrix proteins since only a few proteins in complex I are labeled by IBTP (15). The proteins separated by this approach show a very similar pattern for mitochondria isolated from both control and ethanol treated animals (Figure 2A). A master map for this profile generated from six individual separations identified 50 proteins in common between mitochondria isolated from control and ethanol fed animals (Figure 2B). There was no significant difference in the total protein density between the separations for mitochondria from control and ethanol fed animals. This sub-proteome of the mitochondria is dominated by matrix proteins and indicates that chronic ethanol consumption has little effect on the levels of these proteins (15).
The increased resolution of proteins using 2D gels resulted in the five major IBTP labeled bands resolved on the 1D gels (Figure 1B) being separated into approximately 40 discrete spots (Figure 3A). Moreover, the 2D gel analysis revealed that there was differential IBTP labeling in specific mitochondrial proteins from ethanol-treated animals when compared to controls. Several protein spots immunoreactive for IBTP (Figure 3B) were matched to the corresponding spots within the gel and identified using MALDI-TOF mass spectrometry. The protein spot numbers in Figure 3B correspond to the identified proteins listed in Table 1. The groups of proteins identified as numbers 4 and 7 were found to be isoforms of the same protein. A total of 7 distinct proteins were identified, of which two proteins (circled in Figure 3A), mitochondrial aldehyde dehydrogenase and glucose-regulated protein 78 (GRP78) showed a consistent and reproducible decrease in IBTP labeling in mitochondria isolated from ethanol-treated animals as compared to controls (Table 1). The majority of proteins showed no significant change while two proteins showed higher levels in the mitochondria from ethanol treated animals although this failed to reach significance.

The low $K_m$ aldehyde dehydrogenase (ALDH) is responsible for >90% of acetaldehyde oxidation in liver (31) and contains numerous cysteine residues including three in the active site (9). Because the oxidation or modification of these critical thiol groups may have a negative impact on protein function, the activity of low $K_m$ ALDH in mitochondria was measured. There was a significant decrease in the specific activity of ALDH in mitochondria from ethanol treated
animals (6.51 +/- 0.6 nmol NADH produced/min/mg protein) as compared to control (9.64 +/- 0.61 nmol NADH produced/min/mg protein, p=0.006; n=5) when low concentrations (5 µM) of acetaldehyde were used in the assay. Addition of the reductant β-mercaptoethanol (0.3 mM) did not restore activity of ALDH in the mitochondria from ethanol treated animals to those measured in controls. The inability of a strong thiol reductant to restore ALDH activity indicates that an irreversible oxidative modification to the active site cysteine may have occurred as a consequence of chronic alcohol consumption. Thus, the decrease in activity and IBTP labeling of the low K_m ALDH was due to the oxidation of critical thiols and was not due to a decrease in the amount of the ALDH protein. This was confirmed by measuring the levels of the enzyme by western blotting with an antibody directed against the enzyme which showed no significant difference between the amount of ALDH immunoreactive protein in mitochondria from ethanol or control animals (Figure 3C).

DISCUSSION

Protein thiols are frequent sites for post-translational modification mediated by oxidative or nitrosative stress (6, 22) in a wide variety of disorders including aging, ischemia-reperfusion and alcohol hepatotoxicity (17, 18, 28). An important cellular target for alcohol-induced damage in liver is mitochondria, and it is well known that chronic alcohol consumption adversely affects the structural and functional integrity of hepatic mitochondria (8). Specifically, oxidative stress and generation of ROS/RNS appear to play an important role in alcohol-induced
mitochondrial dysfunction (1, 7). Indeed, previous studies indicated a significant increase in the levels of protein carbonyls in mitochondria from animals fed ethanol chronically (4). However, the identity of specific mitochondrial proteins modified as a consequence of chronic alcohol-induced oxidative stress is not known. Herein we report for the first time the use of IBTP to identify specific mitochondrial proteins that have altered thiol redox status following chronic alcohol consumption.

Mitochondria from ethanol-treated animals are known to have a decreased rate of respiration (8), thus it is possible that the levels of IBTP that accumulated in mitochondria might be different between control and ethanol groups. However, this is probably not the case because Lin et al (15) demonstrated that the uptake of IBTP into mitochondria was dependent on the mitochondrial membrane potential, which is not altered by chronic alcohol consumption alone (20). It was not possible to label mitochondria with IBTP over a wide range of membrane potentials due to limited viability of the mitochondrial preparations. To minimize the impact of the membrane potential, IBTP labeling was performed under conditions of State 3 respiration. Under these circumstances control of respiration is less dependent upon the activity of the electron transport chain. Initial studies using standard 1D SDS-PAGE and immunoblotting for the IBTP protein adduct indicated this was indeed the case and was not significantly altered as a consequence of chronic alcohol consumption (Figure 1). However, the use of 2D electrophoresis revealed that there was differential labeling of several mitochondrial proteins in response to chronic ethanol treatment (Figure
3). It is important to note that of the subset of proteins labeled by IBTP only a limited number showed changes again consistent with a minimal effect of membrane potential on the IBTP available for reaction with thiols. Furthermore, 2D gel electrophoresis allowed the identification of several IBTP-labeled proteins by MALDI-TOF mass spectrometry (Table 1). The low $K_m$ mitochondrial ALDH and the GRP78 consistently showed a significant decrease in IBTP labeling in mitochondria isolated from ethanol-fed rats. GRP78 is a member of the heat shock protein 70 (HSP70) family and is involved in the folding and assembly of proteins in the endoplasmic reticulum (ER) (10). GRP78 has been shown to be an alcohol response gene in a model of alcohol induced hepatotoxicity in mice (12). Even though GRP78 is predominantly localized in the ER, it has been demonstrated in mitochondria (5, 21). However, the functional significance of an alteration in the thiol redox status of GRP78 in the context of chronic alcohol consumption and mitochondrial dysfunction is currently not known.

One of the major proteins showing decreased labeling with IBTP was the low $K_m$ ALDH which resides in the mitochondrial matrix and contributes to the metabolism of acetaldehyde, the oxidation product of ethanol (31) and as such is an important component of cellular defenses against toxic aldehydes (16). In addition there is considerable evidence that chronic ethanol consumption induces lipid peroxidation in the liver, which results in the generation of reactive aldehydes, which are capable of forming adducts with proteins (19, 27). Recent studies have determined that these aldehyde-protein adducts can be immunogenic and as such could potentially contribute to the pathophysiology of
alcoholic liver injury (19). Analysis of low $K_m$ ALDH in the present study showed a significant decrease in the specific activity of this enzyme in mitochondria isolated from ethanol-fed rats as compared to controls, which was not due to a chronic alcohol-related decrease in ALDH protein. It is likely that some of the cysteine residues in ALDH may be particularly susceptible to modification by ethanol-dependent formation of electrophilic reactants including aldehydes or reactive nitrogen species. We postulate that the decrease in low $K_m$ ALDH activity was due to modification of the cysteine residue present in the active site of the enzyme by chronic alcohol-related increases in ROS/RNS. Oxidative modification and inactivation of low $K_m$ ALDH in response to chronic ethanol consumption represents a mechanism that could potentially amplify further production of reactive lipid-derived aldehyde species leading to irreversible injury to mitochondria.

In conclusion, these studies have established a sensitive protocol for the identification of mitochondrial proteins with altered redox thiol status, which occurs following chronic alcohol consumption. Whether these modifications contribute to mitochondrial dysfunction following chronic alcohol exposure is not known. However, emerging evidence strongly suggests that alterations in protein thiol status might have biological relevance and contribute to the pathologies associated with several disease states. This area of research is at an early stage of development, but our demonstration that it is possible to focus on redox changes of an important sub-population of proteins is of great significance for the identification of proteins modified and inactivated by ROS/RNS.
REFERENCES


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FIGURE LEGENDS

Figure 1. One-dimension SDS-PAGE and immunoblotting of IBTP-labeled mitochondria from control and ethanol-fed rats. Mitochondria (1 mg protein) isolated from control and ethanol-fed rats were incubated at 37°C in presence of succinate (15 mM) and ADP (0.5 mM) for 3 min. Where indicated FCCP (1.0 µM) or iodoacetic acid (IAA, 1.0 mM) were added to mitochondria incubations for 1 min, after which mitochondria were centrifuged, the supernatant discarded, and pellet resuspended in 1 ml of fresh respiration buffer containing succinate, ADP and 5 µM IBTP and incubated at 37°C for 10 min. The labeling of thiols was stopped by the addition of 1.0 mM IAA and mitochondria were pelleted by centrifugation. Mitochondrial protein (10 µg) was separated on 10-18% gradient gels by SDS-PAGE. A, Coomassie-blue staining of the IBTP labeled mitochondrial proteins isolated from a representative pair of control and ethanol-fed rats. B, Immunoblots of IBTP-labeled control and ethanol mitochondria probed with anti-triphenylphosphonium (anti-TPP) antiserum. C, shows the quantification of the density of the five major protein bands showing IBTP immunoreactivity in control and ethanol mitochondria (n=6).

Figure 2. High resolution separation of liver mitochondria proteins from control and ethanol fed rats using 2D gel electrophoresis. Mitochondria isolated from control and ethanol-fed rats were separated by 2D electrophoresis, A, For 2D SDS-PAGE, 50 µg mitochondria protein was loaded onto IEF gels (pH gradient 4-8.5) and focused overnight and the second dimension separation was
performed using 8-20% gradient gels. B, Illustrates a master image of all the protein spots detected when gel images were combined and analyzed using PDQuest image analysis software.

**Figure 3. Representative 2D immunoblots from control and ethanol mitochondria demonstrating immunoreactivity against anti-TPP.** Second dimension gels were transferred overnight and nitrocellulose membranes were incubated with a 1:30,000 dilution of the anti-TPP antiserum which was followed by incubation with a 1:5,000 dilution of donkey anti-rabbit HRP conjugated secondary antibody. IBTP-labeled protein thiols were visualized by ECL detection and images were captured using an Alpha Innotech System. A, immunoblots from one pair of control and ethanol mitochondria probed with the anti-TPP antiserum. Aldehyde dehydrogenase and GRP78 are highlighted by circles and indicate proteins that contained significantly less IBTP labeling following chronic alcohol consumption. B, A total of 7 proteins were identified by MALDI-TOF analysis as containing IBTP reactive protein thiols. C, Liver mitochondrial protein (25 µg) from control and ethanol fed rats was separated by SDS–PAGE, transferred to nitrocellulose membranes, and incubated with 1:10,000 anti-rat liver ALDH, followed by incubation with 1:5,000 dilution of donkey anti-rabbit HRP conjugated secondary antibody. Proteins were visualized by ECL detection.
Table 1. Identity, molecular mass, and criteria for identification of proteins spots containing IBTP-reactive thiol groups.

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<th>Protein</th>
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<th>Fold change Ethanol</th>
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Protein spots were identified by position on the western blot and matched to the corresponding protein of the gel of liver mitochondria. The 6th column shows the fold change in protein spot density of ethanol-fed rats when compared to control. Results represent mean ± SE where n=3,*p<0.01
Figure 1

Panel A: Control, Ethanol, FCCP, IAA.

Panel B: Control, Ethanol, FCCP, IAA.

Panel C: Area (arbitrary density units) vs. band number.

- control
- ethanol
Figure 2

A

Control

1D isoelectric focusing

pl values

4.0 8.5

kDa

113
92
52
35
29
18

Ethanol

pl values

4.0 8.5

2D SDS-PAGE

B
Figure 3

A

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Grp78
Aldehyde dehydrogenase

B

C

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