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Alteration of Heart Tissue Protein Profiles in Acute Cadmium-Treated Scallops *Patinopecten yessoensis*

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Abstract Cadmium (Cd) is an extremely toxic metal that induces a wide spectrum of toxic responses in organisms in the environment. In the present study, scallops (*Patinopecten yessoensis*), after acclimation for 1 week in the laboratory, were subjected to acute Cd chloride ($CdCl_2$) toxicity, and ultramorphological and proteomic changes in their heart tissues were analyzed and compared with those of the nonexposed control group. Electron microscopy showed that ultrastructures of the cytoplasm and mitochondria in scallop hearts were badly damaged, and two-dimensional gel electrophoresis showed 32 protein spots that were differentially expressed after exposure to 10 mg/l $CdCl_2$ for 24 h. Of these spots, 8 were upregulated, 16 were downregulated, and 8 showed low expression. Proteins from these spots were identified using matrix-assisted laser desorption/ionization-time of flight mass spectrometry and database searching. The results indicated that these proteins are involved in the regulation of cell structure, transport, signal transduction, and metabolism. Among other things, four proteinsidentified as amino acid adenosine triphosphate (ATP)–binding cassette transporter, glycerol-3-phosphate dehydrogenase (nicotinamide adenine dinucleotide phosphate), nicotinamide adenine dinucleotide oxidase, and ATPasēwere demonstrated to be especially associated with Cd toxicity. Some of the other proteins observed in this work are of particular interest in terms of their responses to Cd, which have not been reported previously. These data may provide novel biomarkers for monitoring the Cd contamination level of flowing seawater as well as provide useful insights into the mechanisms of Cd toxicity.

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Cadmium (Cd) is a nonessential but highly toxic element that is widespread in the environment. Presently, a variety of responses have been attributed to Cd toxicity, but the exact molecular mechanism responsible for the toxic effects of Cd is far from being completely understood. It has been suggested that Cd^{2+} might displace Zn^{2+} and Fe^{2+} in proteins, resulting in their inactivation (Stohs and Bagchi 1995). Quig (1998) proposed that Cd is sulfhydryl reactive. Its toxicity is caused by its strong binding capacity to sensitive groups, such as thiols and histidyls, thus leading to the deterioration of biologically important molecules (Vallee and Ulmer 1972). In addition, Cd may induce oxidative stress by producing hydroxyl radicals (O'Brien and Salasinski 1998), superoxide anion radicals, nitric oxide, and hydrogen peroxide (H_2O_2; Stohs et al. 2001; Waisberg et al. 2003), resulting in lipid peroxidation, DNA damage, and *S*-glutathionylation of proteins (Risso-de Faverney et al. 2001; Géret et al. 2002; Bebianno and Serafim 2003).

Because it is a major water pollutant, Cd can penetrate aquatic organisms and induce detrimental effects (Zyadah and Abdel-Baky 2000; Atli and Canli 2007). Cd taken up from contaminated seawater and transported by way of blood may be harmful to heart tissues of aquatic organisms. Many studies have reported that Cd produces cardiotoxicity (Limaye and Shaikh 1999). It has been demonstrated that Cd can induce disruption of antioxidant defenses in rat heart (Jamall and Smith 1985; Ognjanovi et al. 2006). Pettersen et al. (2002) noted that Cd causes a decrease in

rat heart calcium content. Many cardiovascular diseasesēsuch as dilated cardiomyopathy (Prozialeck et al. 2006), atherosclerosis (Navas-Acien et al. 2005), hypertension (Tomera et al. 1994; Varoni et al. 2003), and myocardial infarction (Everett and Frithsen 2008)ēhave been observed to be associated with blood and urinary Cd in humans. However, the previously mentioned studies were all mammal related, and the effects of Cd induction on the hearts of aquatic organisms, especially marine organisms, have seldom been mentioned.

Therefore, in the present work, we analyzed ultrastructural and proteomic alterations in scallop (*Patinopecten yessoensis*) heart after acute $CdCl_2$ exposure using transmission electron microscopy, two-dimensional gel electrophoresis (2D-GE), and matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) to investigate the effects of Cd on scallop heart and thus shed new light onto the mechanism underlying Cd toxicity. Furthermore, detection and identification of the proteins whose expression levels were altered by Cd will assist in the development of biomarkers for monitoring Cd contamination of seawater and evaluation of the risk of Cd to halobios survival as well as human health.

Materials and Methods

Reagents and Equipment

Carrier ampholyte (pH 5 to 8) was purchased from Amersham Biosciences (Sweden). $CdCl_2$, potassium ferricyanide, acetonitrile, acetone, glutaraldehyde, Epon618, trichloroacetic acid (TCA), trifluoroacetic acid (TFA), acrylamide, bis-acrylamide, urea, thiourea, trihydroxymethyl aminomethane (Tris), dithiothreitol (DTT), 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), iodoacetamide (IAA), and matrix α-cyano-4-hydroxy-cinnamic acid (CHCA) were obtained from Sigma (United States). Sequencing-grade modified trypsin was purchased from Promega (United States). A JEM 2100 (Jeol, Japan) transmission electron microscope (TEM), a DRC-e (PE: Perkin Elmer, United States) inductively coupled plasma (ICP) mass spectrometer, a Reflex III (Bruker, Germany) MALDI-TOF mass spectrometer, an ultracentrifuge (Beckman Coulter, United States), and a vacuum centrifuge (Labconco, United States) were used in this study.

Animals and Treatment

Scallops *P. yessoensis* were purchased from the Xiamen Fish Company, Xiamen, China. After being acclimated for 1 week in the laboratory, six healthy animals of approximately the same size were selected and exposed to 2.5, 5, and 10 mg/l $CdCl_2$ that was prepared using stock seawater. Meanwhile, six animals were bred in stock seawater to serve as the control group. Feeding was suspended during the experiment, and seawater Cd concentration was measured using ICP-MS. After 24 h of exposure, the animals in each group were killed and their heart tissues excised and flushed with physiologic saline to eliminate blood content. If they were not to be used for electron microscopy, the prepared hearts were quickly frozen in liquid nitrogen and stored at −80°C until further use. Three replicate experiments were analyzed for each test concentration as well as the control group, but the experimental results described here are only for the 10 mg/l treatment because this concentration represented acute Cd toxicity better than the other two (2.5 and 5 mg/l) treatments, in which ultrastructure and protein profiles showed only minor changes in scallop heart compared with controls.

Electron Microscopy Analysis

For electron microscopy, freshly prepared hearts were immediately fixed with 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) and postfixed in 1% osmium tetroxide in 0.2 M phosphate buffer (pH 7.2). After postfixation, samples were washed in buffer, dehydrated through an ethanol-acetone series, and embedded in Epon618. Ultrathin sections were cut using an ultracut surfactant. The ultrathin sections were double stained sequentially with uranylacetate and lead citrate before electron microscopy. Images were obtained using a JEM 2100 TEM.

Extraction of Heart Proteins

Frozen heart tissue samples (0.1 g) were homogenized in 5 mL 10% TCA in acetone and stored at −80°C for 4 h. The homogenates were then centrifuged at 12,000×*g* for 10 min. The resulting pellets were washed with acetone to remove residual TCA, resuspended in lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 60 mM DTT, 10 mM Tris, 1 mM ethylene diamine tetraacetic acid, 0.5% carrier ampholyte, and 1% protease-inhibitor cocktail), and placed on ice for 2 h. After sonication, the homogenates were centrifuged at 100,000×*g* for 30 min to remove debris. The remaining supernatants were collected for 2D-GE, and protein content was measured using the Bradford assay. Aliquots of protein samples were kept at −80°C until use.

2D-GE

2D-GE was performed essentially as reported previously (Feng et al. 2008). In brief, 80 μg protein sample was loaded on to 13 cm strips, and isoelectric focusing (IEF)

was performed using carrier ampholyte (pH 5.0 to 8.0) for 10,000 voltage hour. Before sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the gel strips were equilibrated for 15 min in an equilibration buffer (6 M urea, 30% glycerol, 50 mM Tris–HCl, 2% SDS, and 1% DTT [pH 8.8]). The strips were then placed on top of the polyacrylamide gel (T = 12%), and electrophoresis was performed at a constant power of 25 mA/gel until the bromophenol blue reached the bottom of the gel. For each protein sample (control or $CdCl_2$ treatment), three experimental replicates were subjected to 2D-GE.

Silver Staining and Image Analysis

The proteins on the gels were visualized using silver staining as described previously (Zhuo et al. 2007). The silver-stained gels were scanned in a gel documentation system 8000pc imaging system. Digitalized images of the gels were analyzed using ImageMaster 2D Elite software. Protein spots were detected and matched between different samples, and individual spot volume values were obtained according to the program's instructions. To eliminate gel-to-gel variation, the individual spot volume of each gel was normalized relative to the total valid spot volume. The resulting data were exported to Microsoft Excel, where the differentially expressed spots between the control and $CdCl_2$-treatment groups were selected with a criterion of $p < 0.05$ obtained by Student's t test.

In-Gel Digestion

Protein spots were manually cut from the 2D gels with a scalpel, transferred to 500 μl Eppendorf tubes, and destained with destaining solution (30 mM potassium ferricyanide and 100 mM sodium thiosulfate 1:1 [v/v]) until the brown color disappeared. They were rinsed with water, dehydrated with 100% acetonitrile, and dried in a vacuum centrifuge. Proteins were reduced at 56°C for 60 min with 10 mM DTT in 50 mM NH_4HCO_3, followed by alkylation with 55 mM IAA in 50 mM NH_4HCO_3 for 45 min at room temperature in the dark. Gel pieces were washed with 25 mM NH_4HCO_3 for 15 min, dehydrated with 100% acetonitrile, and dried in a vacuum centrifuge. The dried gel pieces were rehydrated with 25 mM NH_4HCO_3 containing 10 ng/μl trypsin for 45 min on ice. After removing the supernatant, 25 mM NH_4HCO_3 was added, and digestion was performed overnight at 37°C. The liquid was collected, and peptides were extracted twice by adding 5% TFA and 50% acetonitrile for 15 min and dried in a vacuum centrifuge.

MALDI-TOF MS Analysis and Database Search

The peptide extracts were redissolved in 5 μl 0.5% TFA, and 1 μl of the peptide mixtures was mixed with an equal volume of matrix CHCA saturated with 50% acetonitrile and 0.1% TFA and spotted onto a MALDI target plate. The samples were then analyzed with a MALDI-TOF mass spectrometer. Peptide mass fingerprints (PMFs) were obtained using reflective and positive ion modes. Laser shots, 200/spectrum, were used to acquire spectra with a mass range of 800 to 3000 Da. Spectra were calibrated using trypsin autodigested ion peaks (m/z 842.510 and 2211.1046) as internal standards. PMF data were used to search for candidate proteins using MASCOT (http://www.matrixscience.com) software. The search parameters were set up as follows: the databases were SWISS-PROT and NCBInr; the minimum number of matched peptides was four; there was fixed modification of carbamidomethylation and variable modification of methionine oxidation; monoisotope masses were used; the number of missed cleavage sites allowed was up to 1; and the maximal mass tolerance was 100 ppm.

Results and Discussion

$CdCl_2$ Altered Structure of Scallop Heart Organelles

The effect of heavy metals on cellular organization is an important factor in understanding the physiologic alterations induced by heavy metals because of their complimentarity of structure and function (Jin et al. 2008). Here, in control scallops, heart cells had typical ultrastructure (Fig. 1a), and dense cytoplasm with well-shaped mitochondria were some of the prominent features in the electron micrographs. The ultrastructural changes began to appear, with obvious differences observed in scallop heart exposed to $CdCl_2$ (Fig. 1b). As can be seen from the electron micrographs, the cytoplasm was less dense and the number of vacuoles increased; cristae in the mitochondria almost disappeared; and detrimental vacuolation even occurred in some of the mitochondria. Previous studies have indicated that Cd treatment of cells results in cytoplasmic and mitochondrial alterations (Dudley et al. 1984; Toury et al. 1985; Jin et al. 2008; Daud et al. 2009). In the current work, the ultramorphological changes that occurred in scallop heart indicated severe Cd damage to scallops, and this could provide evidence for the subsequent analysis of stress proteins induced by Cd exposure.

$CdCl_2$ Altered Scallop Heart Protein Profiles

To analyze the scallop heart proteome, samples were subjected to 2D-GE analysis. Approximately 600 protein spots in each gel were visualized using silver staining (Fig. 2), and most spots were distributed in the pH range of 5 to 8. The profiles of the two maps were very similar, suggesting highly reproducible 2D-GE in the current study.

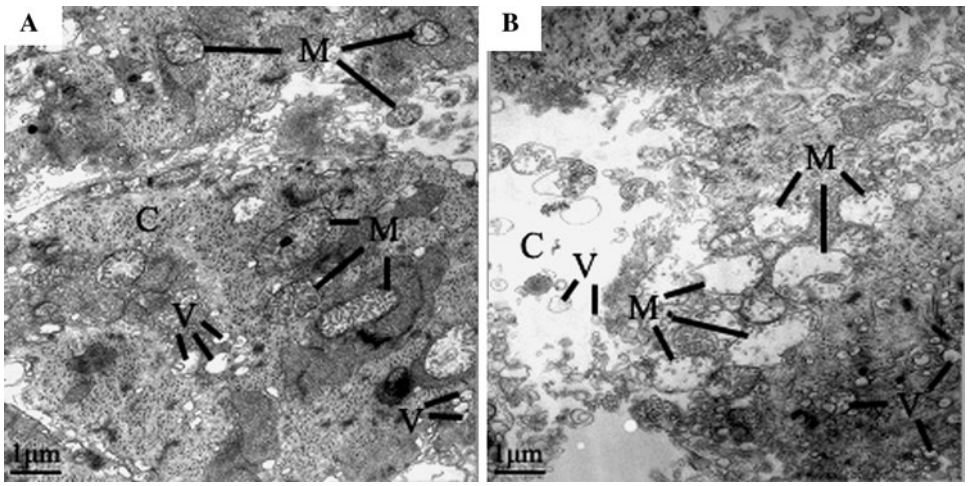

Fig. 1 Electron micrographs of heart tissue in scallops from the **a** control and **b** $CdCl_2$-treatment groups exposed to 10 mg/l $CdCl_2$ for 24 h. *C* cytoplasm, *M* mitochondria, *V* vacuole. Original magnification 5000×

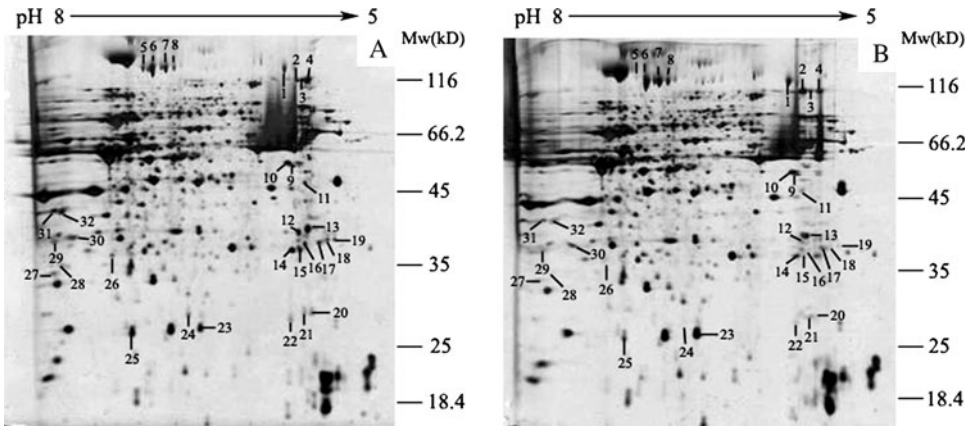

Fig. 2 2D-GE patterns of total protein spots from the scallop heart samples from the **a** control and **b** $CdCl_2$-treatment groups. Total heart proteins (80 μg) were loaded and separated using IEF (pH 5–8)/SDS-PAGE (12% acrylamide). Gels were silver stained. Spots marked with *numbers* indicate proteins with a modified expression level after exposure to 10 mg/l $CdCl_2$ for 24 h

Comparison between the control (Fig. 2a) and $CdCl_2$-treatment groups (Fig. 2b) using ImageMaster 2D Elite software showed 32 protein spots, which displayed a statistically significant change in the experimental group with respect to the control group, and they are marked as nos. 1 to 32 in Fig. 2. Among these 32 altered spots, 8 (nos. 1, 2, 3, 6, 7, 8, 10, and 23) were upregulated (Fig. 3a); 16 (nos. 4, 9, 11, 12, 13, 14, 16, 17, 18, 20, 25, 27, 29, 30, 31, and 32) were downregulated (Fig. 3b); and 8 (nos. 5, 15, 19, 21, 22, 24, 26, and 28) showed low expression (Fig. 3c).

Identification of Differential Proteins

The differential protein spots shown in Fig. 3 were further identified using MALDI-TOF MS and database searching after an in-gel digestion procedure. The analytic and research results are listed in Table 1, and the proteins identified were divided into clusters according to their functional properties by searching the Gene Ontology data base (http://www.geneontology.org/) (Fig. 4). All of the major functional groups were represented, including proteins involved in binding, catalysis, cell structure, redox regulation, metabolism, transport regulation, and signal transduction. Previous reports have shown that Cd toxicity is relevant to many processes, such as metabolism, oxidative stress, signaling pathways, DNA damage, transcriptional regulation, apoptosis, and carcinogenesis (Fernandez et al. 2003; Lau and Chiu 2007; Matz and Krone 2007; He et al. 2008; Yu et al. 2008). In the present research, multiple types and functions of the differential proteins induced by $CdCl_2$ exposure also indicated the complexity of Cd toxicity.

Differentially Expressed Proteins Already Associated with Cd Stress

Among the differentially expressed proteins, four were considered to be related to Cd toxicity as previously reported (Table 2). In fact, a decreased in abundance was observed for amino acid ATP–binding cassette (ABC) transporter, NADH oxidase (NOX) and ATPase, and even glycerol-3-phosphate dehydrogenase (GPDH) [$NAD(P)^+$] showed a low level of expression under acute Cd stress conditions. The subcellular localization of these proteins were analyzed (Table 2) by searching the UniProt database (http://www.uniprot.org/). GPDH and ATPase were located in the cytoplasm, whereas amino acid ABC transporter and

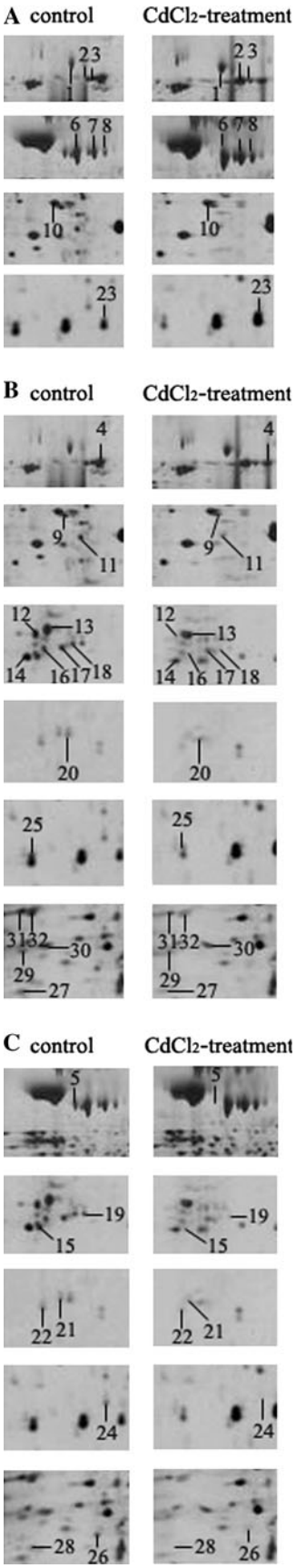

Fig. 3 Magnified images of protein spots that showed significantly ▶ different changes between the control and $CdCl_2$-treatment groups. **a** Upregulated proteins found in the $CdCl_2$-treatment group with respect to the control group. **b** Downregulated proteins found in the $CdCl_2$-treatment group with respect to the control group. **c** Low-expression proteins found in the $CdCl_2$-treatment group with respect to the control group

NOX were located in the mitochondria. This suggests that the metabolic pathways of Cd^{2+} in scallop heart occurred primarily in the cytoplasm and mitochondria. These results were in accord with the previously observed phenomenon in this work that the cytoplasm and mitochondria structures were badly damaged in Cd^{2+}-exposed scallops.

ABC Transporters

ABC transporters are membrane-integrated proteins that translocate chemical substrates across membranes with the consumption of energy by ATP hydrolyzation. It is known that ABC transporters participate in absorption, accumulation, and excretion of various toxicants and thus play an important role in the proactive defense of organisms (Bovet et al. 2005; Nagya et al. 2006). Previous data have shown that MRP1-4 can roll out the Cd^{2+}-chelate complex or GS–Cd complex across vacuole membranes to decrease Cd content in the cell (Rea et al. 1998). Here, the expression level of amino acid ABC transporter was downregulated by $CdCl_2$ treatment, which indicates the weakened detoxification capability of the Cd-poisoned scallops. In addition, decreased expression of the ABC transporter has been observed in response to Cd^{2+} in *Paralichthys olivaceus* (Ling et al. 2009). Rustichelli et al. (2008) demonstrated that ABC transporters are down-expressed in *Physcia adscendens* after Cd exposure.

GPDH [NAD(P)$^+$]

$NAD(P)^+$-linked GPDH is localized in the cytoplasm and uses $NAD(P)^+$ as its electron acceptor. GPDH occupies the branch point between the glycolytic pathway and triglyceride biosynthesis and carries out a number of distinct metabolic functions, including (1) generation of glycerol-3-phosphate for phospholipid and triglyceride synthesis; (2) conversion of dihydroxyacetone phosphate to glycerol-3-phosphate during gluconeogenesis from glycerol; and (3) maintenance of cytosolic NAD^+ level during anaerobic glycolysis (Bissell et al. 1976). Berrada et al. (2002) noted that Cd^{2+} can inhibit GPDH activity in skeletal muscles of jerboa (*Jaculus orientalis*). In addition, Zn^{2+} has been observed to inhibit the activity of rabbit muscle GPDH (Maret et al. 2001). In our current research, GPDH showed a low level of expression n scallop heart induced by $CdCl_2$ exposure, suggesting the adverse effects of Cd^{2+} on glycerol, lipid, and energy metabolism of the organism.

Table 1 Identification of proteins differentially expressed in response to acute exposure of at 10 mg/l $CdCl_2$ for 24 h

Spot No.	Accession no.[a]	Sequence coverage (%)[b]	Mw (Da)/pI[c]	Description (protein name)	Relative intensity[d]	
					Control	Cd treated
1	gil27366593	8	136691/5.16	Signal-transduction histidine kinase	0.1280 ± 0.0206	0.2210 ± 0.0158
2	P18091-01-00-00	11	107636/5.48	Splice isoform nonmuscle from P18091, alpha-actinin, sarcomeric	0.1160 ± 0.0439	0.3000 ± 0.0559
3	P10567	10	102002/5.28	Paramyosin	0.1080 ± 0.0342	0.0809 ± 0.0105
4	gil68380883	11	130759/6.36	Predicted: Similar to inducible nitric oxide synthase	0.2740 ± 0.0503	0.1740 ± 0.0221
6	gil66804747	10	1362925.59	Actin-binding protein	0.4800 ± 0.1026	0.5300 ± 0.0281
7	gil47220070	11	149992/6.75	Unnamed protein product	0.2100 ± 0.0625	0.4090 ± 0.0503
8	gil71040098	9	119649/4.75	Rho GTPase-activating protein 30 isoform 1	0.0748 ± 0.0174	0.1940 ± 0.0451
9	gil68563086	26	76788/5.98	Flavoprotein mono-oxygenase: Mono-oxygenase, FAD binding	0.0929 ± 0.0202	0.0288 ± 0.0113
10	Q91YX0	9	75186/5.85	Induced by contact to basement membrane 1 protein homolog	0.1850 ± 0.0511	0.2370 ± 0.0556
11	gil28851706	21	40814/9.34	Amino acid ABC transporter, permease protein	0.0748 ± 0.0210	0.0513 ± 0.0136
12	gil28807265	25	38146/5.35	Lipoprotein-34 NlpB	0.1490 ± 0.0619	0.0302 ± 0.0098
13	gil75703218	26	37265/6.46	ABC transporter like	0.2700 ± 0.0580	0.2290 ± 0.0283
14	gil71847726	40	36090/8.85	Phosphate-binding protein	0.1910 ± 0.0430	0.1130 ± 0.0329
15	O67555	17	36181/7.10	Glycerol-3-phosphate dehydrogenase [$NAD(P)^+$]	0.1610 ± 0.0550	–
16	gil45656860	13	36541/8.61	Cytochrome c peroxidase	0.1210 ± 0.0220	0.0327 ± 0.0141
17	gil26339952	24	43333/8.59	Unnamed protein product	0.1080 ± 0.0430	0.0527 ± 0.0268
18	gil71369622	14	42015/8.89	NADH oxidase	0.0837 ± 0.0329	0.0346 ± 0.0087
19	gil72063018	26	39780/6.04	Predicted: Similar to CG10802-PA, partial	0.0574 ± 0.0212	–
20	P63652	22	27547/5.97	Putative CoA-transferase beta subunit Rv3552/MT3656	0.0828 ± 0.0386	0.0740 ± 0.0405
21	Q6XYT0	26	30492/9.61	Phosphate import ATP-binding protein pstB	0.0556 ± 0.0128	–
22	Q73JU8	18	28945/8.61	Methyltransferase gidB	0.0650 ± 0.0198	–
23	Q9HK42	20	24663/5.52	UPF0173 metal-dependent hydrolase Ta0764	0.2080 ± 0.0120	0.4990 ± 0.0173
24	gil11496832	39	28474/5.37	Outer surface protein D (ospD)	0.0572 ± 0.0145	–
25	gil7331197	20	37970/8.39	Putative purple acid phosphatase precursor	0.3130 ± 0.0899	0.1520 ± 0.0.383
26	gil27380890	28	42443/9.99	Hypothetical protein blr5779	0.0627 ± 0.0102	–
27	gil25058739	24	73381/6.42	ALB protein	0.1050 ± 0.3587	0.0469 ± 0.0071
28	gil62528004	34	33783/6.78	Predicted phosphohydrolases	0.0568 ± 0.0062	–
29	gil76883936	29	43429/5.81	GTP-binding signal-recognition particle SRP54	0.1420 ± 0.0321	0.1080 ± 0.0425
30	P26560	23	38678/6.22	VP7 core protein	0.2420 ± 0.0590	0.1280 ± 0.0412
31	O31777	13	43313/6.24	2-amino-3-ketobutyrate coenzyme A ligase (AKB ligase)	0.1560 ± 0.0305	0.0700 ± 0.0205
32	gil42517483	27	36877/8.31	ATPase	0.2390 ± 0.0710	0.1090 ± 0.0215

[a] Protein accession numbers came from SWISS-PROT database or NCBInr Data Bank

[b] Percentage of the protein sequence covered by the matched peptides

[c] Predicted molecular weight and pI of the protein in the databases

[d] Relative volumes (individual/total) of corresponding spots (shown as mean ± SD) in 2D-GE gel of control and $CdCl_2$-treatment groups. *Dashes* indicate that spots could not be detected in 2D-GE gel of the $CdCl_2$-treatment group

pI isoelectric point

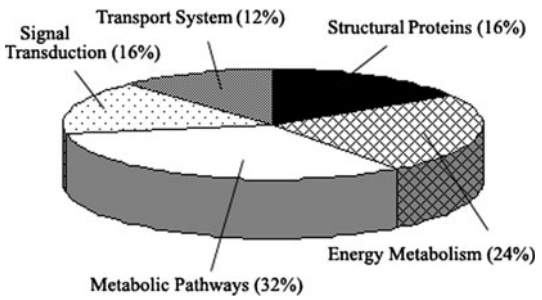

Fig. 4 Functional clustering of identified differential proteins showing relative representation (indicated as percentage of all the identified proteins) for each protein family

NOX

NOX is a flavoprotein that catalyzes the oxidation of NADH to NAD^+ and reacts with oxygen to produce either water or hydrogen peroxide. Thus, the NOXs differ regarding their products, i.e., H_2O- and H_2O_2 -forming enzymes (Park et al. 1992; Ross and Claiborne 1992). NOX is an important component of the aerobic respiratory chain. In this work, decreased NOX abundance was observed. Beard et al. (1995) reported that Cd(II) inhibits NOX activity in the membranes of *Escherichia coli* K-12 and proposed that Cd(II) inhibits NOX activity by binding to at least two sites in the respiratory chain. Copper is also considered to be a metal inhibitor of NOX (Gaisser et al. 1987; de Zwart et al. 1991). Because the enzyme was inhibited, electron transport and energy production in the respiratory chain must have been hampered, and thus we propose that Cd^{2+} represses oxidative phosphorylation in scallop heart.

ATPase

ATPase is an integral membrane enzyme that catalyzes ATP hydrolysis and supplies energy directly for life activities. There are many types of ATPases in organisms, among which Na^+ K^+-ATPase, Ca^{2+}-ATPase, and Mg^{2+}-ATPase are the three major ones found in aquatic organisms. They have important functions, such as ion transport, maintenance of electrochemical gradient, and regulation of cell volume (Canli and Stagg 1996; Grosell et al. 2003). Many studies have suggested that Cd affects ATPase activity in aquatic organisms. Atli and Canli (2007) note that gill and intestine Na^+ K^+-ATPase and muscle Ca^{2+}-ATPase activities are inhibited by Cd in *Oreochromis niloticus*. Na^+ K^+-ATPase enzymatic activities, as measured in intestinal and branchial of eel (*Anguilla anguilla*), are inhibited by Cd in a dose-dependent manner (Lionetto et al. 2000). Na^+, K^+-, Mg^{2+}-, and Ca^{2+}-ATPase activities all decrease progressively with the length of Cd exposure in muscle of carp fry and fingerlings (*Cyprinus carpio*) (Suresh et al. 1995). Thevenod and Friedmann (1999), in their study of the effects of Cd on rat proximal tubule cells, proposed that oxidative damage caused by Cd increases degradation of Na^+ and K^+-ATPase through the proteasomal and endo/lysosomal proteolytic pathways. In our research, ATPase expression was decreased in Cd^{2+}-exposed scallop heart, which is consistent with the previously mentioned reports.

Novel Differential Proteins Associated with Cd Stress

Apart from the proteins reported with reference to Cd toxicity, some proteins were observed to be modulated and were of particular interest in terms of their responses to Cd. Although these modulations have never been described, they included cytochrome *c* peroxidase (CcP), phosphate-import ATP-binding protein pstB, and methyltransferase gidB. CcP oxidizes H_2O_2 to water using cytochrome *c* as the electron donor and protects cellular components from oxidative damage (Erman and Vitello 2002). Here, CcP was downregulated by Cd treatment, indicating that Cd causes depletion of antioxidants and leads to oxidative damage in scallops. Phosphate-import ATP-binding protein

Table 2 Subcellular localization of representative proteins associated with $CdCl_2$ toxicity

Spot no.	Protein name	Functional description	Subcellular localization[a]	Reference[b]
11	Amino acid ABC transporter, permease protein	Part of the binding protein–dependent transport system for an amino acid	Mitochondria	Rustichelli et al. (2008)
15	Glycerol-3-phosphate dehydrogenase [NAD(P)$^+$]	Catalyzes production of glycerol under anaerobic growth conditions	Cytoplasm	Berrada et al. (2002)
18	NADH oxidase	Catalyzes the four-electron reduction of molecular oxygen to water	Mitochondria	Beard et al. (1995)
32	ATPase	Catalyzes the hydrolyzation of ATP and produces energy	Cytoplasm	Suresh et al. (1995)

[a] Cellular component in which the protein was located

[b] Previous reference reporting that the protein was associated with Cd toxicity

PstB is a catalytic subunit that couples the energy of ATP hydrolysis to the import of phosphate across cellular membranes through the phosphate-specific transporter (Pst) system (Surin et al. 1985; Higgins 2001). In this study, a much lower level of PstB was found in Cd-exposed scallops, which indicates the adverse effects of Cd on phosphate transport. Methyltransferase gidB is an enzyme that specifically methylates the N7 position of guanosine in position 527 of 16S rRNA. This enzyme exhibited a low level of expression in Cd-treated scallop heart. This shows that blocking of 16S rRNA methylation may play an important role in contributing to Cd toxicity.

Conclusion

The comparative analysis described here showed significantly altered expression levels for various proteins in heart tissue of Cd-exposed scallops. These proteins were used in part as biomarkers indicating Cd toxicity, although evidence of the relation between their modulation and Cd stress is still lacking. In addition, some proteins were proposed as novel biomarkers to further explore the mechanisms of Cd toxicity. We hope these results may provide positive insights for a better understanding of Cd toxicology as well as the identification of useful biomarkers for the evaluation and monitoring of Cd contamination in aquatic environments.

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