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Cadmium-Detoxification in the Earthworm *Enchytraeus:* Specific Expression of a Putative Aldehyde Dehydrogenase

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The ubiquitous earthworm *Enchytraeus buchholzi* is provided with a very effective, but still unknown, mechanism of Cadmium (Cd)-detoxification, a central role in which has been ascribed to the Cd-inducible *crp*-gene encoding a novel cysteine-rich, non-metallothionein 25 kDa protein. This study identifies another Cd-responsive gene by differential screening of a cDNA-library constructed from Cd-exposed *E. buchholzi.* The isolated cDNA-clone designated Ebaldh encodes a putative aldehyde dehydrogenase. Northern blot analysis shows that the *Ebaldh*-expression is strongly enhanced by Cd, but remains unaffected by other stressors such as Zn, Hg, and H_2O_2. This Cd-specificity of *Ebaldh* suggests that Cd-detoxification in *E. buchholzi* requires low intracellular concentrations of aldehydes which are known to target sulfhydryl groups thus inactivating the Cd-binding capacity of CRP.

The pollution of the environment by Cadmium (Cd) is increasingly realized as a hazard for plants, animals and even for human health. The worldwide input of Cd into the biosphere is estimated to about 30 000 tons/year with more than 90 % coming from anthropogenic sources (1). Moreover, the sustained acidification of the environment due to the acid rain solubilizes the immobilized Cd and, thus, increases the bioavailability of Cd (2). Nevertheless, some organisms, as e.g. the earthworm *Enchytraeus buchholzi*, can survive in acidic soils highly contaminated with Cd. These small, about 2-15 mm long oligochaetes are highly abundant in various terrestrial habitats including forrested and cultivated areas (3). Toxicity tests indicate that they tolerate Cd-concentrations up to 3.6 g/kg dry weight in soil (4) and up to 8 mg/l in artificial aqueous media, respectively (5). This high Cd-tolerance involves a novel cysteine-rich, non-metallothionein 25-kDa-protein, designated CRP (6). Remarkably, the gene encoding CRP is *de novo* expressed when the worms begin to accumulate Cd (6,7). CRP is obviously an essential part of a novel detoxification mechanism for Cd. However, it is not unlikely that this Cd-detoxification process involves other genes and proteins, respectively, not yet identified. This prompted us to search for other Cd-responsive genes at the mRNA-level by differential screening of a cDNA-library constructed from Cd-exposed *E. buchholzi.*

MATERIALS AND METHODS

Animals. Mass cultures of the oligochaete *Enchytraeus buchholzi* were grown in artificial soil at 20°C in darkness. The experiments were carried out in a fluid medium consisting of 1 g $Ca(NO_3)$, 0.25 g $MgSO_4$, 0.25 g KNO_3, 0.25 g KH_2PO_4 and traces of $FeSO_4$ per 1 l H_2O at pH 5.0 adjusted with K_2HPO_4 as detailed recently (6,7).

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The nucleotide sequence reported in this paper has been submitted to the EBI Data Library with accession number X95396.

Abbreviations: LC_{50}, median lethal concentration; NOEC, no observed effect concentration; ALDH, aldehyde dehydrogenase (EC 1.2.1.3).

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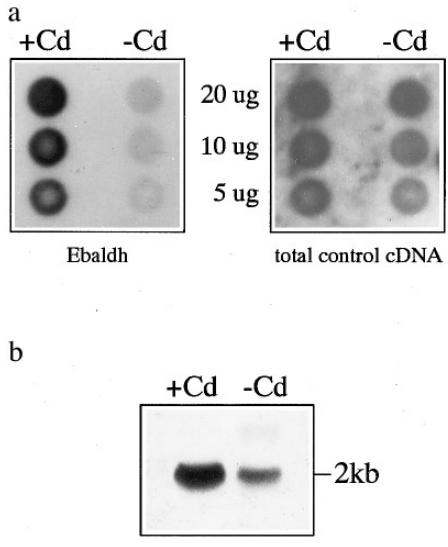

FIG. 1. Expression of the Ebaldh-mRNA. The worms were incubated for 6 days in a fluid medium without Cd (−Cd) and with 3 mg Cd/l (+Cd), respectively, before extracting RNA. (a) Dot blot analysis. Total RNA of the indicated amounts was denatured with formaldehyde, blotted onto a nylon membrane and hybridized with the Ebaldh-cDNA probe. After autoradiography, the RNA was rehybridized with a cDNA-probe synthesized from control poly(A)$^+$-RNA by reverse transcription. (b) Northern blot analysis. Poly(A)$^+$-RNA (5 μg/lane) was denatured with glyoxal, separated on an agarose gel and blotted onto a nylon membrane before hybridization with the Ebaldh-cDNA probe.

Toxicity tests. The LC_{50}-values and No-Observed-Effect-Concentrations (NOEC) of Cd, Hg, Zn and H_2O_2 were determined by exposing at least 20 mature worms of the same size (3-4 mm) to different concentrations of each stressor for 2 days as described recently (7).

RNA extraction. Total RNA was isolated (8), and poly(A$^+$)-RNA was prepared by standard oligo(dT)-cellulose chromatography (9).

Differential screening. A cDNA-library in λZAP XR vector (Stratagene, Heidelberg, Germany) was prepared from worms exposed to the sublethal Cd-concentration of 3 mg/l for 6 days and, then, differentially screened as recently described in detail (6).

Dot- and northern-blot analysis. For dot blots, total RNA was denatured with formaldehyde and formamide before blotting onto a Nylon-membrane (Nytran, Schleicher and Schuell, Dassel, Germany) as described by Sambrook *et al.* (10). For northern blots, total RNA or poly(A$^+$)-RNA were denatured with glyoxal and separated in 1% agarose gels (11) and blotted onto nylon membrane filter. Dot- and northern blots were hybridized at 65°C for 16 h in 6× SSC, 0.5% SDS, and 100 μg/ml salmon sperm DNA with [^{32}P]-labeled DNA probes synthesized by random priming from the appropriate cDNA. After washing in 0.2× SSC, 0.1% SDS for 1 h at 65°C, the filters were exposed to Kodak X-Omat films. After autoradiography, the dot blots were washed in 1 mM Tris, 1 mM EDTA at 75°C for 3 h and rehybridized using a cDNA-probe synthesized from poly(A$^+$)-RNA of untreated worms.

DNA sequencing. E. buchholzi cDNA cloned in the pBluescript SK vector was sequenced on both strands by the didesoxynucleotide chain termination method (12) using the T7 sequencing kit (Pharmacia, Freiburg, Germany). Sequence specific oligonucleotides (Birsner and Grob, Denzlingen, Germany) were used as primers for sequencing of the total cDNA-insert. Sequence data were analyzed with the PC/Gene software (Version 6.85, Intelligenetics, Mountain View, CA, U.S.A.). EMBL databases were used to compare DNA and deduced protein sequences.

In vitro transcription and translation. Approximately 2 μg of the appropriate cDNA was transcribed *in vitro* according to the Ambion MaxiScript protocol (Ambion, Austin, U.S.A.) using T3 RNA polymerase. The RNA was extracted with phenol/chloroform and then *in vitro* translated using the Promega rabbit reticulocyte lysate system (Promega, Heidelberg, Germany) and the ICN translabel mixture containing [^{35}S]methionine and [^{35}S]cysteine (>1000 Ci/mmol, ICN). About 10 % of the [^{35}S]-labeled translation products were separated by 12% SDS-PAGE (13) and then fluorographed (14).

RESULTS

The cDNA-library constructed from worms exposed to 3 mg Cd/l for 6 days was differentially screened using Cd-induced and control first strand cDNA-probes. Screening of approxi-

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CGAATTGTAAGTTACGCTGTTAAAATTAAACATAAAGTTCTCTACAAAACTTTAGTTTGCAACGAACTTGAC   72
AGTCGTCGTACCAGTAATCCATCAAAAATGTCCGCTCCTAAGATTCCCGAGCCCGTGAAAGATCTTAAAGTG  144
                              M  S  A  P  K  I  P  E  P  V  K  D  L  K  V
GAATTTACGAAGATATTCATAAACAACGAGTTTGTGGACTCGGTGAGCGGCAAGACATTCGCCACTATCAAC  216
E  F  T  K  I  F  I  N  N  E  F  V  D  S  V  S  G  K  T  F  A  T  I  N
CCGTCGACAGGAGAGAAGCTTGCTGAGGTTCAGGAGGGCGACAAGGCAGATATAGACAAGGCAGTGGCGGCC  288
P  S  T  G  E  K  L  A  E  V  Q  E  G  D  K  A  D  I  D  K  A  V  A  A
GCTAGGGCAGCCTTCAAGAGAGATGCTGAGTACAGGAAGCACGACGCTTCTGACAGAGGGCGCCTCTTGTTC  360
A  R  A  A  F  K  R  D  A  E  Y  R  K  H  D  A  S  D  R  G  R  L  L  F
AAGCTCGCTGATCTCATCGAGGCTCACAGAGTTCAGCTACGTACGCTCGAGACACTGGACAATGGCAAGCCA  432
K  L  A  D  L  I  E  A  H  R  V  Q  L  R  T  L  E  T  L  D  N  G  K  P
TTCGCCATGTCGTACCTGGGTGACACTCTGATGGCACAGAAGGTTCTGAGGTACTACGCTGGGTTTGCTGAT  504
F  A  M  S  Y  L  G  D  T  L  M  A  Q  K  V  L  R  Y  Y  A  G  F  A  D
AAGATCGTAGGGCAGACTATTCCAGCTGATGGTAACGTGTTCTGCTACACAAGACACGAGCCTGTGGGCGTG  576
K  I  V  G  Q  T  I  P  A  D  G  N  V  F  C  Y  T  R  H  E  P  V  G  V
GTCGGAGCTATCACACCCTGGAACTTTCCCCTGCACTTGGCGGCCTCTAAGATTGCTCCAGCCATTGCTGCG  648
V  G  A  I  T  P  W  N  F  P  L  H  L  A  A  S  K  I  A  P  A  I  A  A
GGCTGTACGCTTGTGCTCAAACCTGCTGAACAGACACCGCTAACTGCTCTCTATCTCGCCTCACTCGTCAAA  720
G  C  T  L  V  L  K  P  A  E  Q  T  P  L  T  A  L  Y  L  A  S  L  V  K
CAGGCGGGGTTTCCAGCAGGTGTTATTAACATAGTGCCAGGTCTGGGTCACACAGCAGGTGCTGCTCTCACC  792
Q  A  G  F  P  A  G  V  I  N  I  V  P  G  L  G  H  T  A  G  A  A  L  T
AACCATCCTGACATCAACAAGATAACCTTCACTGGCTCCACTGAGGTGGGACAGTTGATCATCCAGGGATCA  864
N  H  P  D  I  N  K  I  T  F  T  G  S  T  E  V  G  Q  L  I  I  Q  G  S
GGCAAGACAAACCTGAAGAGAGTGACTCTGGAGCTGGGTGGAAAGAGTCCCAATATCATCTTCCCGGACTCT  936
G  K  T  N  L  K  R  V  T  L  E  L  G  G  K  S  P  N  I  I  F  P  D  S
GACTTGGACTATGCGGTGGAGGTGTCCCACCAGGCTATCATGGCTAACATGGGTCAGGTCTGCTGCGCTGGG 1008
D  L  D  Y  A  V  E  V  S  H  Q  A  I  M  A  N  M  G  Q  V  C  C  A  G
TCGAGAACATTCGTGCACGAGGACATCTACGAGGAATTTGTGCGTAGGAGCGTGGAGCGAGCCAAGAAGAGA 1080
S  R  T  F  V  H  E  D  I  Y  E  E  F  V  R  R  S  V  E  R  A  K  K  R
ACTGTCGGTGACCCCTTCGATCCCAAGAACGAGAATGGACCCCAGGTTGATGAGACTCAGTTGAAAAAGATT 1152
T  V  G  D  P  F  D  P  K  N  E  N  G  P  Q  V  D  E  T  Q  L  K  K  I
CTGGAGCTGATAGAGTCCGGTAAGACGGAGGGGGCTAAGCTGGAGTGTGGAGGGAAGAGACTCGGAGACAAG 1224
L  E  L  I  E  S  G  K  T  E  G  A  K  L  E  C  G  G  K  R  L  G  D  K
GGATACTTCGTCGAGCCCACCGTGTTCACTGACGTCACCAGCAGCATGAGGGTCGCCAAGGAGGAGATCTTT 1296
G  Y  F  V  E  P  T  V  F  T  D  V  T  S  S  M  R  V  A  K  E  E  I  F
GGCCCAGTTCAGCTGATCTTCAAGTTCAAGGACGTGGACGAGGTCATAGAGAGAGCCAACGACACAAGCTAC 1368
G  P  V  Q  L  I  F  K  F  K  D  V  D  E  V  I  E  R  A  N  D  T  S  Y
GGGCTGGCTGCGGCTGTCTTCACCAAGAACATCGACACAGCCCTCAAAGTGGCCAACAGTCTGGAGGCAGGC 1440
G  L  A  A  A  V  F  T  K  N  I  D  T  A  L  K  V  A  N  S  L  E  A  G
ACCGTCTGGGTGAACACGTACAACCACTTTGCTTTCCAGGCTCCATTCGGTGGATACAAGATGTCAGGACAG 1512
T  V  W  V  N  T  Y  N  H  F  A  F  Q  A  P  F  G  G  Y  K  M  S  G  Q
GGCCGTGAATTCGGACATTACGGACTCGAAGCCTTCCTAGAAGTAAAGACTGTGTACGTGCGCACTCCTACC 1584
G  R  E  F  G  H  Y  G  L  E  A  F  L  E  V  K  T  V  Y  V  R  T  P  T
AAACTGTGAACAATCGGCTGAGCATCTGAGCTGAACGTTAAGCAAGTCAACTTAAACCAGTGCTAAACTATT 1656
K  L  -
ACAACCTAGAGCGGTGCTTATTGACATGCAATAACTGACACTCATGAAACCTATGCTGTGCTTGTGACATCT 1728
GTCGAATGAAATTAATGTTTTCTTATTTACTGGACTCTACATGTGCTGCCATCTCACTGAGATTAAATCTAG 1800
TTTTGTGGAGTTTTATACCGCCTGTGGACCAGTTTCTTGAGTTAATTGTTCTGCATTGATTGTGTGGCGATC 1872
TGTTAGAATTGAAACGGGTGAAATTTGTACTGTTAAGAGATTAAAATATTGTCAAGATACTAAAATAGTATT 1944
AATTAAAGTTAAATTCAGGTGTTTTGTTGTGCTGCAGTTGCCGGTTTTCTCTTATTTCTTCTAATAAAGCAT 2016
TTACGATCAATCAAAAAAAAAAAAAAAAAA
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FIG. 2. Full-length sequence of the Ebaldh-cDNA and deduced amino acid sequence. The polyadenylation signal at position 2007 is underlined.

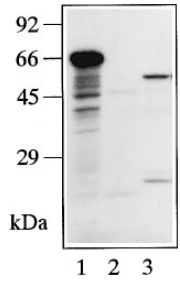

FIG. 3. *In vitro* translation products of the Ebaldh-cDNA. The Ebaldh-cDNA was first transcribed *in vitro* and the resulting RNA was then translated in a rabbit reticulocyte lysate using [^{35}S]methionine and [^{35}S]cysteine. The translation products were separated on a 12% SDS-polyacrylamide gel and detected by fluorography. *Lane 1:* Control with luciferase RNA; *lane 2:* Control without RNA; *lane 3:* Translation products of a Ebaldh-cDNA.

mately 3×10^5 recombinants of the amplified library resulted in the identification of a Cd-induced cDNA-clone, designated Ebaldh. The differential expression of Ebaldh-mRNA was verified by dot blot analysis of total RNA prepared from Cd-exposed and control worms (Fig. 1a). This analysis revealed that the *Ebaldh*-gene is constitutively expressed, but its expression is strongly enhanced in Cd-exposed worms. This conclusion is further substantiated by northern blot analysis using poly(A)$^+$-RNA (Fig. 1b).

The complete sequence of Ebaldh-cDNA has a length of 2048 bp (Fig. 2). It represents very likely the full-length copy of the Ebaldh-mRNA since its corresponding mRNA is also about 2 kb in size (Fig. 1b). The Ebaldh-cDNA contains an open reading frame of 1493 bp beginning with the first ATG-codon at position 101. The polyadenylation signal is located 16 bp upstream from the poly(A)-tail. There is a 64 % identity of the Ebaldh-nucleotide sequence with the cDNA of the cytosolic human aldehyde dehydrogenase (ALDH) without any gaps inserted in both sequences.

The deduced amino acid sequence of EbALDH comprises 497 amino acids (Fig. 2). The predicted molecular mass of 54 kDa fits that determined experimentally by *in vitro* translation of *in vitro* transcribed Ebaldh-cDNA. SDS-PAGE of the translation products revealed a dominant band at about 54 kDa (Fig. 3). Additionally, some minor bands appeared at lower molecular masses, which may arise from endogenous mRNAs of the reticulocyte lysate and internal ATG-codons of the Ebaldh-mRNA, respectively. The EbALDH amino acid sequence reveals a 61 % identity to the human cytosolic ALDH (Fig. 4). Moreover, the EbALDH contains all the sequence motifs which are highly conserved in the ALDHs known to date. These are the FINNE-motif near the N-terminus, the VTLELGGKSP-motif around the glutamic acid active site and the EEIFGPV-motif near the C-terminus (15). Furthermore, a glutamic acid is located at position 266 and a cysteine at position 300 of the EbALDH, which both are part of the active site of ALDHs (16,17).

We also investigated other stressors such as Hg, Zn and H_2O_2 for their capacity to enhance the expression of the Ebaldh-mRNA. The effect of the different stressors was determined by northern blot analysis of total RNA extracted from worms which were exposed to NOEC of each stressor for 2 days (Fig. 5). Surprisingly, the expression of *Ebaldh* is only increased after exposure of the worms to Cd, whereas Hg, Zn, and H_2O_2 did not alter *Ebaldh*-gene expression at all.

DISCUSSION

This study shows that exposure of the earthworm *E. buchholzi* to subtoxic Cd-concentrations results in a strongly enhanced expression of a gene designated *Ebaldh*. This encodes

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Enchytr                          MSAPKIPEPVKDLKVEFTKIFINNEFVDSVSGKTFA    36
HumanCyt                      SSSGTPDLPVLLTDLKIQYTKIFINNEWHDSVSGKKFP    38
HumanMit   MLRAAARFGPRLGRRLLSAAATQAVPAPNQQPEVFCNQIFINNEWHDAVSRKTFP    55
SquidOc                          AGIPPPTRNPEIKHNKIFINNQFVNSTSNKAYP    33
                                 . .* .  ....  ..*****......*.*..

Enchytr    TINPSTGEKLAEVQEGDKADIDKAVAAARAAFKRDAEYRKHDASDRGRLLFKLAD    91
HumanCyt   VFNPATEEELCQVEEGDKEDVDKAVKAARQAFQIGSPWRTMDASERGRLLYKLAD    93
HumanMit   TVNPSTGEVICQVAEGDKEDVDKAVKAARAAFQLGSPWRRMDASHRGRLLNRLAD   110
SquidOc    VYNPCDNEKVCEVQEGDKSDIDKAVQACKAAFKRGTPWRRMDASRLGHLLYRLAD    88
           . **...*.. .*.**** *.**** *...**..    *  *** .*.** .***

Enchytr    LIEAHRVQLRTLETLDNGKPFAMSYLGDTLMAQKVLRYYAGFADKIVGQTIPADG   146
HumanCyt   LIERDRLLLATMESMNGGKLYSNAYLNDLAGCIKTLRYCAGWADKIQGRTIPIDG   148
HumanMit   LIERDRTYLAALETLDNGKPYVISYLVDLDMVLKCLRYYAGWADKYHGKTIPIDG   165
SquidOc    LMERDIAYMASLETMDTGKPYKNSY-QDMVHCIQVLRYFAGWADKNMGESIPVDG   142
           *.*  .  . ..*....**.   .*. *  .  ..***.** ***. * .** **

Enchytr    NVFCYTRHEPVGVVGAITPWNFPLHLAASKIAPAIAAGCTLVLKPAEQTPLTALY   201
HumanCyt   NFFTYTRHEPIGVCGQIIPWNFPLVMLIWKIGPALSCGNTVVVKPAEQTPLTALH   203
HumanMit   DFFSYTRHEPVGVCGQIIPWNFPLLMQAWKLGPALATGNVVVMKVAEQTPLTALY   220
SquidOc    DFFCYTRNEPVGVCGLLIPYNYPMLMMTWKMAPALACGNCMIVKPAEQTPLTALY   197
           . *.***.**.** * . *.*.*.   . *..** . * . . *.*********.

Enchytr    LASLVKQAGFPAGVINIVPGLGHTAGAALTNHPDINKITFTGSTEVGQLIIQGSG   256
HumanCyt   VASLIKEAGFPPGVVNIVPGYGPTAGAAISSHMDIDKVAFTGSTEVGKLIKEAAG   258
HumanMit   VANLIKEAGFPPGVVNIVPGFGPTAGAAIASHEDVDKVAFTGSTEIGRVIQVAAG   275
SquidOc    CASLIKEAGFPPGVVNVVPGFGKICGQYISSHQEINKVSFTGSTEVGMLVMQDAG   252
            *.* * **** ** *.*** * ..*..   * ...*  ******.* .. .  *

Enchytr    KTNLKRVTLELGGKSPNIIFPDSDLDYAVEVSHQAIMANMGQVCCAGSRTFVHED   311
HumanCyt   KSNLKRVTLELGGKSPCIVLADADLDNAVEFAHHGVFYHQGQCCIAASRIFVEES   313
HumanMit   SSNLKRVTLELGGKSPNIIMSDADMDWAVEQAHFALFFNQGQCCCAGSRTFVQED   330
SquidOc    KSNLKRCSMQLSGKCPLVVFEDTELDFAVQQAHEAAFQNMGQCRWSGSRTYVHEN   307
           . ****....*.**.*.... * ..* **.  * .   ..** ....**..*.*.

Enchytr    IYEEFVRRSVERAKKRTVGDPFDPKNENGPQVDETQLKKILELIESGKTEGAKLE   366
HumanCyt   IYDEFVRRSVERAKKYILGNPLTPGVTQGPQIDKEQYDKILDLIESGKKEGAKLE   368
HumanMit   IYDEFVERSVARAKSRVVGNPFDSKTEQGPQVDETQFKKILGYINTGKQEGAKLL   385
SquidOc    IYDEFVKRAVEKATSRKTGDPYEMDTEHGPQIDEEQYKKIMEFIKKGKDKGAQLK   362
           ** ***.*.*..*... .*.*.... . ***.*..* .**...*..** .**.*.

Enchytr    CGGKRLGDKGYFVEPTVFTDVTSSMRVAKEEIFGPVQLIFKFKDVDEVIERANDT   421
HumanCyt   CGGGPWGNKGYFVQPTVFSNVTDEMRIAKEEIFGPVQQIMKFKSLDDVIKRANNT   423
HumanMit   CGGGIAADRGYFIQPTVFGDVQDGMTIAKEEIFGPVMQILKFKTIEEVVGRANNS   440
SquidOc    CGGNRHGDKGFYVEPTVFSDVTDEMKFSQEEIFGPVQLIMKFKDMDEVIDRCNNT   417
           *** . ...*....**** .*.  *. ..*******..* ***. ..*. *.* .

Enchytr    SYGLAAAVFTKNIDTALKVANSLEAGTVWVNTYNHFAFQAPFGGYKMSGQGREFG   476
HumanCyt   FYGLSAGVFTKDIDKAITISSALQAGTVWVNCYGVVSAQCPFGGFKMSGNGRELG   478
HumanMit   TYGLAAAVFTKDLDKANYLSQALQAGTVWVNCYDVFGAQSPFGGYKMSGSGRELG   495
SquidOc    DYGMAAAIFTNDINRSITFTHAMYCGTVWVNTYNHWFPQAPFGGYKKSGLYREMG   472
            **..*..**. .. .      . .******.*...  *.****.*.** .** *

Enchytr    HYGLEAFLEVKTVYVRTPTKL    497
HumanCyt   EYGFHEYTEVKTVTVKISQKNS   500
HumanMit   EYGLQAYTEVKTVTVKVPQKNS   517
SquidOc    KYTLQEYTEVKNIVYRIPQKNS   494
            *.. .  ***.. .. . *
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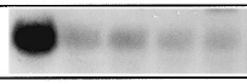
	Cd	Zn	Hg	H_2O_2	contr.
	[mg/l]			(ppm)	
LC_{50}	8.4	57	0.14	27	--
NOEC	4.0	30	0.04	10	--
Ebaldh at NOEC					

FIG. 5. Expression of Ebaldh-mRNA after exposure of *E. buchholzi* to different stressors. The worms were exposed for 2 days at NOEC of each stressor and without stressor as a control (contr.). Northern blot analysis was done using 20 μg total RNA/lane and the Ebaldh-cDNA as hybridization probe.

a 54 kDa protein which reveals over 61 % sequence identity with cytosolic human ALDH (19,20). As the latter, EbALDH does not possess a signal sequence, in contrast to human mitochondrial ALDH (Fig. 4). In other metazoan invertebrates, sequence data of ALDHs are not available to date. The only exception is the omega-crystallin which is the major structural protein of eye lenses of squids and octopi (20). The omega-crystallins are closely related to the mammalian ALDHs as revealed by sequence comparisons (Fig. 4), but have lost their enzymatic activities. ALDHs catalyze the irreversible oxidation of aldehydes to their corresponding acids.

Many mammalian *aldh*-genes are known to be inducible by a variety of stressors (15). To our knowledge, however, *aldhs* have not yet been described to be inducible by Cd. Surprisingly, the *Ebaldh* is specifically stimulated by Cd. Other heavy metals such as Hg and Zn as well as the reactive oxygen species H_2O_2 did not affect *Ebaldh* expression at all. This specific effect of Cd on *Ebaldh* expression does therefore not reflect a general stress response, but rather suggests a specific role of *Ebaldh* in the peculiar Cd-detoxification process in *E. buchholzi.* This view is also supported by the fact that the *Ebaldh* expression correlates with the Cd-inducible expression of the *crp*-gene. Indeed, the Cd-induced increase in *Ebaldh* expression occurs at that time when maximal expression of *crp* is induced by Cd (6,7).

The *crp*-gene encodes a novel cysteine-rich, non-metallothionein protein which is essential for the detoxification of Cd in *E. buchholzi* (6,7). The CRP-protein consists mainly of 8 repeats arranged tandemly, each composed of 31 amino acids. These repeats are very similar to the heavy metal binding domains of mammalian metallothioneins, which have about the same number and arrangement of cysteine residues (21). The sulfhydryl groups of cysteines presumably detoxify Cd via thiolate bonds. In this context, it is noteworthy (i) that Cd *per se* generates aldehydes, for example, via lipid peroxidation (22), and (ii) that aldehydes are toxic due to preferably targeting sulhydryl groups. It is therefore an attractive speculation that the effective Cd-detoxification in *E. buchholzi* requires

FIG. 4. Comparison of EbALDH amino acid sequence with sequences of human cytosolic (HCytALDH) and mitochondrial ALDHs (HMitALDH) and the omegacrystallin (OMCRYST) of the squid *Ommastrephes sloanei.* The sequence motifs which are conserved in all known ALDHs are written in boldface. The glutamic acid residue and the cysteine residue at the active site are written in italics (pos. 266 and pos. 300 of EbALDH). The mitochondrial signal peptide is underlined. Asterisks indicate amino acid residues which are perfectly conserved in all sequences. Points indicate the identity of an EbALDH amino acid residue with at least one of the amino acid residues of the other sequences.

EbALDH to lower intracellular concentrations of aldehydes, thus protecting the Cd-binding capacity of sulfhydryl groups of CRP.

REFERENCES

1. Nriagu, J. O., and Pacyna, J. M. (1988) *Nature* **333,** 134–139.
2. Stoeppler, M. (1991) *in* Metals and Their Compounds in the Environment (Merian, E., Ed.), pp. 803–855, Verlag Chemie, Heidelberg/New York.
3. Didden, W. A. M. (1993) *Pedobiologia* **37,** 2–29.
4. Roembke, J. (1989) *Arch. Toxicol. Suppl.* **13,** 402–405.
5. Willuhn, J., Schmitt-Wrede, H.-P., Greven, H., and Wunderlich, F. (1994) *Ecotoxicol. Environ. Saf.* **29,** 93–100.
6. Willuhn, J., Schmitt-Wrede, H.-P., Greven, H., and Wunderlich, F. (1994) *J. Biol. Chem.* **268,** 24688–24691.
7. Willuhn, J., Otto, A., Schmitt-Wrede, H.-P., and Wunderlich, F. (1996) *Biochem. Biophys. Res. Comm.* **220,** 581–585.
8. Chirgwin, J., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
9. Aviv, H., and Leder, P. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69,** 1409–1412.
10. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
11. McMaster, G. K., and Carmichael, G. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74,** 4835–4840.
12. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74,** 5463–5467.
13. Laemmli, U. K. (1970) *Nature* **227,** 680–685.
14. Chamberlain, J. P. (1979) *Anal. Biochem.* **98,** 132–135.
15. Lindahl, R. (1992) *Crit. Rev. Biochem. Mol.* **27,** 283–335.
16. Wang, X., and Weiner, H. (1995) *Biochemistry* **34,** 237–243.
17. Farrés, J., Wang, T. T. Y., Cunningham, S. J., and Weiner, H. (1995) *Biochemistry* **34,** 2592–2598.
18. Hempel, J., von Bahr-Lindstrom, H., and Jornvall, H. (1984) *Eur. J. Biochem.* **141,** 21–28.
19. Hsu, L. C., Chang, W.-C., Shibuya, A., and Yoshida, A. (1992) *J. Biol. Chem.* **267,** 3030–3037.
20. Zinovieva, R. D., Tomarev, S. I., and Piatigorsky, J. (1993) *J. Biol. Chem.* **268,** 11449–11455.
21. Hamer, D. H. (1986) *Ann. Rev. Biochem.* **55,** 913–951.
22. Sarkar, S., Yadar, P., Trivedi, R., Bausal, A. K., and Bhatnangar, D. (1995) *J. Trace Elem. Med. Biol.* **9,** 144–149.