



Gene cloning and mRNA expression of glutamate dehydrogenase in the liver, brain, and intestine of the swamp eel, *Monopterus albus* (Zuiew), exposed to freshwater, terrestrial conditions, environmental ammonia, or salinity stress

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The swamp eel, *Monopterus albus*, is an obligatory air-breathing teleost which can undergo long period of emersion, has high environmental and tissue ammonia tolerance, and can survive in brackish water. We obtained a cDNA sequence of glutamate dehydrogenase (*gdh*), which consisted of a 133-bp 5' UTR, a complete coding sequence region spanning 1629 bp and a 3' UTR of approximately 717 bp, from the liver, intestine, and brain of *M. albus*. The translated Gdh amino acid sequence had 542 residues, and it formed a monophyletic clade with *Bostrychus sinensis* Gdh1a, *Tetraodon nigroviridis* Gdh1a, *Chaenocephalus aceratus* Gdh1a, *Salmo salar* Gdh1a1 and Gdh1a2, and *O. mykiss* Gdh1a. One day of exposure to terrestrial conditions or 75 mmol l⁻¹ NH₄Cl, but not to water at salinity 20, resulted in a significant increase in mRNA expression of *gdh1a* and Gdh amination activity in the liver of *M. albus*. However, exposure to brackish water, but not to terrestrial conditions or 75 mmol l⁻¹ NH₄Cl, led to a significant increase in the mRNA expression of *gdh1a* and Gdh amination activity in the intestine. By contrast, all the three experimental conditions had no significant effects on the mRNA expression of *gdh1a* in the brain of *M. albus*, despite a significant decrease in the Gdh amination activity in the brain of fish exposed to 75 mmol l⁻¹ NH₄Cl for 6 days. Our results indicate for the first time that the mRNA expression of *gdh1a* was differentially up-regulated in the liver and intestine of *M. albus* in response to ammonia toxicity and salinity stress, respectively. The increases in mRNA expression of *gdh1a* and Gdh amination activity would probably lead to an increase in glutamate production in support of increased glutamine synthesis for the purpose of ammonia detoxification or cell volume regulation under these two different environmental conditions.

Keywords: ammonia, glutamate, glutamate dehydrogenase, *Monopterus albus*, nitrogen metabolism, osmoregulation, mRNA expression, swamp eel

INTRODUCTION

The swamp eel, *Monopterus albus* (Zuiew) belongs to Order Synbranchiformes, Family Synbranchidae, and is commonly found in muddy ponds, swamps, canals, and rice fields in India, Malaysia, Indonesia, and Southern China (Rainboth, 1996). It is an obligate air breather and its gills are vastly atrophied to become a fold of skin within the opercular chamber (Graham, 1997). As an air breather, *M. albus* can survive on land for an extended period. Since no water is available to flush branchial or cutaneous surfaces during emersion, ammonia excretion becomes inefficient leading to the accumulation of ammonia within the body (Ip et al., 2004b). During drought, ammonia excreted into a small volume of external medium would increase in concentration which may reach to levels that would impede endogenous ammonia excretion. For fish living in the rice field, it may encounter high levels of environmental ammonia (Ip et al., 2004a) during the application of

ammonium sulfate fertilizers (Freney et al., 1981). Since the environmental salinity may increase when the habitat dries up during drought or during high tide in swamps located at the river mouth, *M. albus* may also encounter hyperosmotic stress.

Ammonia is toxic to fishes as it affects many cellular processes (Ip et al., 2001, 2004a,b; Chew et al., 2006; Ip and Chew, 2010), but some tropical air-breathing teleosts can ameliorate ammonia toxicity through a variety of adaptations (see Ip et al., 2001, 2004a,b; Chew et al., 2006 for reviews). During 6 days of emersion (Tay et al., 2003) or 40 days of aestivation in mud (Chew et al., 2005), *M. albus* demonstrates high tissue ammonia tolerance, suppresses endogenous ammonia production, and detoxifies endogenous ammonia to glutamine in extra-cranial tissues. Furthermore, *M. albus* has high tolerance of acute ammonia toxicity and detoxifies exogenous ammonia to glutamine (Tng et al., 2009). After 6 days of exposure to 75 mmol l⁻¹ NH₄Cl, ammonia

(6.5–15.2 $\mu\text{mol g}^{-1}$), and glutamine (9.4–17.1 $\mu\text{mol g}^{-1}$) accumulate to very high levels in the brain, muscle, liver, and intestine (Ip et al., 2004c). Hence, glutamine can be regarded as a temporary product of ammonia detoxification in *M. albus* during emersion or ammonia exposure. The fate of glutamine during prolonged exposure to or recovery from emersion or environmental ammonia is unclear at present, but one of the advantages of producing glutamine is that it can be stored momentarily in the body and used for the synthesis of other molecules, e.g., purines and pyrimidines, when the adverse environmental conditions subside. Besides, glutamine can also act as an intracellular osmolyte for the purpose of cell volume regulation. Recently, Tok et al. (2009) reported that *M. albus* switched from hyperosmotic hyperionic regulation in freshwater to a combination of osmoconforming and hypoosmotic hypoionic regulation to survive in brackish water (salinity 25). Salinity stress resulted in relatively large increases in the plasma osmolality, $[\text{Na}^+]$ and $[\text{Cl}^-]$ in *M. albus*. This in turn led to the accumulation of organic osmolytes and inorganic ions for cell volume regulation, with the hepatic glutamine content increased to a phenomenal level of $>30 \mu\text{mol g}^{-1}$.

Glutamine synthesis is catalyzed by glutamine synthetase (GS) and requires glutamate as a substrate. Although transamination reactions can generate glutamate, the key enzyme regulating ammonia and glutamate levels in vertebrates is glutamate dehydrogenase (GDH; Shoemaker and Haley, 1993), which is involved in several metabolic pathways (Hudson and Daniel, 1993; Thatcher and Storey, 2001). GDH catalyzes both glutamate formation in the amination direction using ammonia and α -ketoglutarate as substrates and glutamate degradation in the deamination direction (Smith et al., 1975; Hudson and Daniel, 1993). To date, there is a dearth of information on how adverse conditions would affect the mRNA expression of *gdh* in various tissues of *M. albus* and in fish in general. Therefore, this study was undertaken to clone and sequence the cDNA of *gdh* from the liver, brain, and intestine of *M. albus*. Efforts were then made to determine whether exposure to terrestrial conditions, environmental ammonia (75 mmol l^{-1} NH_4Cl), or brackish water (salinity 20) would induce changes in the mRNA expression of *gdh* in these three organs.

MATERIALS AND METHODS

FISH

Specimens of *M. albus* (150–400 g body mass) were purchased locally from a fish farm in Singapore. They were maintained in plastic aquaria in freshwater (salinity 1) at 25°C under a 12 h:12 h dark:light regime in the laboratory and water was changed daily. No attempt was made to separate the sexes. Fish were acclimated to laboratory conditions for at least 1 week before experimentation, during which they were fed live guppy. Food was withdrawn 48 h prior to experiments, which gave sufficient time for the gut to be emptied of all food and waste products. Fish were not fed throughout the experimental period. Procedures adopted in this study were approved by the Institutional Animal Care and Use Committee of the National University of Singapore (IACUC 021/10).

EXPOSURE OF FISH TO EXPERIMENTAL CONDITIONS AND COLLECTION OF SAMPLES

Fish were exposed to control or experimental conditions individually in plastic tanks (50 cm length \times 30 cm width \times 10 cm height) with free access to air. A total of 12 fish were immersed in 10 volumes (w/v) of freshwater and they served as controls. Water was changed daily. For terrestrial conditions, fish were kept in plastic aquaria tanks containing a thin film (100 ml) of freshwater. These tanks were rinsed and the freshwater was replenished daily. For ammonia exposure, a total of 8 fish were immersed in 10 volumes of freshwater containing 75 mmol l^{-1} NH_4Cl (pH 7), which was also changed daily. For salinity stress, 4 fish were immersed in 10 volumes (w/v) of water of various salinities prepared by mixing freshwater with an appropriate quantity of seawater, and the pH was adjusted to 7. The ambient salinity was raised progressively through a 4-day period from freshwater (day 0) to salinity 5 (day 1), salinity 10 (day 2), salinity 15 (day 3), and salinity 20 (day 4). At the end of the exposure periods (day 1, 4, and 6 for control fish, $N = 4$ each; day 1 and 6 for fish exposed to terrestrial or ammonia conditions, $N = 4$ each; day 4 for fish exposed to salinity stress, $N = 4$), fish were killed with a strong blow to the head, and samples of the liver, intestine, and brain were excised, frozen in liquid nitrogen, and stored at -80°C until analysis.

TOTAL RNA EXTRACTION, cDNA SYNTHESIS, PCR, AND GENE SEQUENCING

The total RNA was extracted from the liver, intestine, and brain, using the chaotropic extraction protocol described by Whitehead and Crawford (2005), and purified using RNeasy Mini Kit (QIAGEN Inc., Hilden, Germany). RNA quality was checked electrophoretically and quantified at 260 nm using a 105.810-UVS Hellma traycell (Hellma GmbH & Co., KG, Müllheim, Baden-Württemberg, Germany) adapted to a Shimadzu UV-1601 UV-VIS recording spectrophotometer (Shimadzu Corporation, Nakagyo-ku, Kyoto, Japan). The total RNA (1 μg) from each sample was then reverse transcribed using RevertAidTMM-MuLV Reverse Transcriptase and oligo(dT)₁₈ primer from the RevertAidTMFirst Strand cDNA Synthesis Kit (Fermentas Inc., Hanover, MD, USA). PCR was performed on the cDNA obtained using the forward primers 1, 2, and 3 and the reverse primers 4, 5, 6, and 7 (Table 1). The primer sequences for 1 and 4 were obtained from Hirata et al. (2003) while the primer sequences for 2, 3, 5, 6, and 7 were obtained from Peh (2008). Each PCR was carried out in 25 μl reaction volumes, containing 10 \times Dreamtaq Buffer (2.5 μl ; Fermentas Inc.), dNTPs (10 $\mu\text{mol l}^{-1}$, 0.5 μl), MgCl_2 (25 mmol l^{-1} , 0.5 μl), Dreamtaq polymerase (5 IU μl^{-1} , 0.125 μl ; Fermentas Inc.), forward primer and reverse primers (10 $\mu\text{mol l}^{-1}$, 1.25 μl each), and cDNA template (0.5 μl), using a Veriti[®] Fast 96-well Thermal Cycler (Applied Biosystems Inc., Foster City, CA, USA). The following thermocycling program was used: 95°C for 3 min, followed by 40 cycles of 30 s at 94°C, 30 s at 55°C, and 90 s at 72°C and 1 cycle of final extension at 72°C for 10 min. The PCR products obtained were separated by 1% agarose gel electrophoresis and the band of expected size was excised from the gel. The PCR product was purified from the gel slice by the centrifugation method of Wizard SV gel and PCR clean-up system kit

Table 1 | Primers for PCR or RACE PCR to amplify glutamate dehydrogenase (*gdh*), or for quantitative real-time PCR on actin (reference gene) and *gdh 1a* from the liver, intestine, and brain of *Monopterus albus*.

Primer	Primer sequence	Amplicon size (bp)
DEGENERATE PRIMERS FOR PCR		
Pair 1	1 5'-ATGACNTAYAARTGYGCNGT-3'	1182
	5 5'-TYATGTGAMGGTRAKVCCVGCCTC-3'	
Pair 2	2 5'-ATGTAYMGRTAYTTCGGRGAR-3'	679
	4 5'-GCRANTGTRTCNGCDATCCA-3'	
Pair 3	3 5'-GACCCMAACTTCTTYMRVATGG-3'	1030
	6 5'-CCYTCWGCRRATRATCTTRGC-3'	
Pair 4	3 5'-GACCCMAACTTCTTYMRVATGG-3'	1165
	7 5'-CRTGRTTHAGRTTCTTBAGC-3'	
PRIMERS FOR RACE PCR		
5 RACE	5'-ATCTTGTGACATCTGGGGTGGTG-3'	
3 RACE	5'-CCACCCAGATGCTGACAAGAT-3'	
PRIMERS FOR SEQUENCING PRODUCTS FROM RACE PCR		
S1	5'-TGGCTGATGGGTTTTCTGTCACAC-3	
S2	5'-TTGGACTIONTACCTGTGA-3'	
PRIMERS FOR QUANTITATIVE REAL-TIME PCR		
<i>actin</i>	5'-CCGTGACCTCACAGACTACCTC-3'	136
F1		
<i>actin</i>	5'-CCATCTCTGCTCGAAGTCCA-3'	
R1		
<i>gdh</i> F1	5'-GCACAGCCAACACAGGACAC-3'	119
<i>gdh</i> R1	5'-GGCACATCCACAACAGCACAC-3'	

(Promega Corporation, Madison, WI, USA). Purified PCR products were subjected to cycle sequencing using BigDye[®] Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems Inc.) and purified by the ethanol/sodium acetate precipitation. Purified products were automatically sequenced using the Prism[™]3130XL Genetic Analyzer (Applied Biosystems Inc.).

RACE PCR AND CLONING

Total RNA (1 µg) was reverse transcribed into cDNA for RACE PCR using SMARTer[™]RACE cDNA Amplification kit (Clontech Laboratories, Mountain View, CA, USA). RACE PCR was performed using the Advantage[®] 2 PCR kit (Clontech Laboratories) with gene-specific *gdh* RACE primers (Table 1). Thermocycling conditions were: 5 cycles at 94°C for 30 s, 72°C for 4 min, continued with 5 cycles at 94°C for 30 s, 70°C for 30 s, 72°C for 4 min, and then followed by 30 cycles at 94°C for 30 s, 68°C for 30 s, 72°C for 4 min. RACE PCR products were separated by 1% agarose gel electrophoresis and the distinct bands observed were excised from the gel. The RACE PCR products were purified from the gel slices and quantified as stated above.

RACE PCR products were cloned using pGEM Easy T/A cloning kit (Promega Corporation), transformed into *Escherichia coli* JM109 strain, and plated onto Luria-Bertani agar with ampicillin, 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside and isopropyl-beta-thio galactopyranoside. Subsequently, white colonies were selected and grown overnight

in LB broth with ampicillin. The plasmids were extracted using the resin-based plasmid miniprep kit (Axygen Biosciences, Union City, CA, USA) and quantified by spectrophotometry. Cloned *gdh* RACE PCR products were then sequenced.

PHYLOGENETIC ANALYSIS

The translated Gdh sequence from *M. albus* was aligned with 25 other Gdh protein sequences using ClustalX version 2.0.12 (Larkin et al., 2007). The sequences used for comparison and their respective accession number in either GenBank or Ensembl databases were as follows: *Oncorhynchus mykiss* Gdh1a (AAM73777.1) and Gdh1b (AAM73775.1), *Danio rerio* Gdh1a (NP_955839.2) and Gdh1b (NP_997741.1), *Salmo salar* Gdh1a1 (CAD58714.1), Gdh1a2 (CAD89353.1), and Gdh1b (CAD58715.1), *Tribolodon hakonensis* Gdh1a (BAD83654.1), *Chaenocephalus aceratus* Gdh1a (P82264.1), *Litopenaeus vannamei* Gdh (ACC95446.1), *Xenopus laevis* GDH1 (NP_001087023.1), *X. (Silurana) tropicalis* GDH1 (NP_001011138.1), *Gallus gallus* GDH1 (P00368.1), *Rattus norvegicus* GDH1 (NP_036702.1), *Mus musculus* GDH1 (NP_032159.1), *Bos taurus* GDH1 (AAI03337.1), *Homo sapiens* GLUD1 (NP_005262.1) and GLUD2 (NP_036216.2), *Takifugu rubripes* Gdh1a (ENSTRUP00000000720) and Gdh1b (ENSTRUP000000009100), *Tetraodon nigroviridis* Gdh1a (ENSTNIP00000008014) and Gdh1b (ENSTNIP00000016349), and *Taeniopygia guttata* GDH1 (ENSTGUP00000005951). Protein sequences for *B. sinensis* Gdh1a and Gdh1b were obtained from Peh (2008). A number of the current gene and protein names in the databases do not reflect co-orthologous relationships to the human gene or paralogous relationships. Therefore, the author has attempted to reflect these relationships by renaming (where applicable) the protein names presented in the phylogenetic tree following the method suggested by Taylor et al. (2001). The PHYLIP package (version 3.5) was then used to carry out neighbor-joining phylogenetic analysis (Saitou and Nei, 1987), with a bootstrapping resampling option to assess the support for nodes (100 pseudoreplicates). The Pacific white shrimp *L. vannamei* was specified as the outgroup and the input order of species was randomized where possible.

QUANTITATIVE REAL-TIME PCR (qPCR)

The primers used for qPCR (Table 1) were designed using Perl-Primer version 1.1.18 (Marshall, 2004). Primers for the housekeeping gene *actin* (Table 1) were designed based on the *M. albus actin* sequence (AY647143.1) deposited in GenBank. All primer pairs produced amplicons of the predicted size. The PCR products from each primer pair were subjected to 2% agarose gel electrophoresis to confirm amplification specificity. The bands were excised from the gel and PCR products purified, quantified, and then sequenced as described above.

Total RNA samples ($N = 4$ for each organ and each condition) were subjected to DNase treatment using deoxyribonuclease I (Sigma-Aldrich Co. St. Louis, MO, USA). Total RNA (1 µg) from each treated sample was then reverse transcribed using random hexamer primers and the RevertAid[™]First Strand cDNA Synthesis Kit (Fermentas Inc.). The qPCR reactions were performed in triplicates using 1 ng of cDNA template, $0.4 \mu\text{mol l}^{-1}$ of each primer, and 5 µl of Fast SYBR[®] Green Master Mix (Applied

Biosystems Inc.) in a volume of 10 μ l. Reactions were analyzed on a StepOnePlus™ Real-Time PCR System (Applied Biosystems Inc.) and cycling conditions were: 95°C for 20 s, 50 cycles at 95°C for 3 s followed by 60°C for 30 s. A melt curve analysis was carried out after each run with the following conditions: 95°C for 15 s, increasing temperature from 60 to 95°C in 1 h, ending with 95°C for 15 s. No primer dimers were detected. Relative standard curves were generated by 10-fold serial dilutions of cDNA from

the liver, intestine, and brain of *M. albus* exposed to freshwater conditions. Amplification efficiencies of *gdh1a* and *actin* ranged between 85 and 90%. The amplification efficiencies of the target (*gdh1a*) and reference gene (*actin*) must be approximately equal for the $\Delta\Delta$ Ct calculation to be valid (Livak and Schmittgen, 2001). Thus, plots of the log cDNA dilution versus Δ Ct were made and the absolute values of the slopes (−0.05 for liver; 0.03 for intestine; 0.06 for brain) were verified to be close to zero.

1	ACTC GGC CAG AGG ACG GGA AAC GGT GAG AAG CTG CCG TCC TTT ATT	46	1172	GGA GCC AAA CCC TAT GAA GGA AAC ATT TTG GAA GCC GAT TGC CAT	1216
47	CTG CCC CCT CTC CTG TCA TCA CCT AAT GGT CCT TGT CGT TTA GTT	91	347	G A K P Y E G N I L E A D C H	361
92	GCT ATT TTA TTT CTT CCT TTA CTC GGT TAG CTA GTA TCA GTC	CDS start → 136	1217	ATC CTG ATC CCA GCT GCT GGT GAA AAG CAG CTT ACA CGT AAC AAT	1261
		ATG M	362	I L I P A A G E K Q L T R N N	376
137	TAT CGA TAT TTT GGA GAA CTG CTG AGC CGC TCG GCG GGT AGT GCC	181	1262	GCC CCC AGG ATC AAG GCC AAG ATC ATT GCT GAA GGT GCC AAT GGT	1306
2	Y R Y F G E L L S R S A G S A	16	377	A P R I K A K I I A E G A N G	391
182	CTG GCC TCG GGG TGC GTG GAT TCC GCT CTC CCC TTG TCC TCG TCC	226		3RACE GDH1 → ← 5RACE GDH1	
17	L A S G C V D S A L P L S S S	21	1307	CCC ACC ACC CCA GAT GCT GAC AAG ATC TTC CTG GAG AAC AAC GTC	1351
227	CTG ATG CGG CTC CGT CAC TAT GCC GAC GCA GCG GAG CAG CCT GAC	271	392	P T T P D A D K I F L E N N V	406
32	L M R L R H Y A D A A E Q P D	46	1352	ATG GTT ATT CCT GAC ATG TAC CTG AAT GCT GGA GGT GTG ACG GTA	1396
272	GAC CCC AAC TTC TTC AAG ATG GTC GAG GGG TTT TTC GAT CGT GGC	316	407	M V I P D M Y L N A G G V T V	421
47	D P F K M V E G G T T C G R G	61	1397	TCT TAT TTT GAG TGG CTC AAA AAT CTC AAC CAT GTT AGC TAC GGA	1441
317	GCA TCC ATC GTG GAG GAC AAG CTG GTG GAG GAT CTG AAA ACC AGG	361	422	S Y F E W L K N L N H V S Y G	436
62	A S I V E D K L V E D L K T R	76	1442	AGA CTG ACC TTC AAA TAT GAG AGA GAC TCA AAC TAC CAC CTG CTC	1486
362	GAG AGC CCC GAG CAG AAG AGG AAC CGT GTG CGG GGC ATC CTG CGT	406	437	R L T F K Y E R D S N Y H L L	451
77	E S P E Q K R N R V R G I L R	91	1487	ATG TCT GTG CAG GAG AGT TTA GAA AGA AAG TTT GGC AAA CAG GGA	1531
407	ATC ATC AAG CCT TGT AAT CAC GTC TTG AGT GTG TCT TTC CCG ATC	451	452	M S V Q E S L E R K F G K Q G	466
92	I I K P C N H V L S V S F P I	106	1532	GGA CCC ATC CCC ATC GTC CCT ACT GCT GAC TTC CAG GCC AGA GTT	1576
452	AAG AGG GAT AGT GGA GAG TGG GAG GTG GTG GAG GGG TAC CGC GCC	496	467	G P I P I V P A D F Q A R V	481
107	K R D S G E W E V V E G Y R A	121	1577	GCT GGT GCT TCT GAG AAG GAT ATT GTT CAC TCT GGG TTG GCC TAC	1621
497	CAG CAC AGC CAA CAC AGG ACA CCT TGC AAA GGA GGT ATC CGT TAC	541	482	A G A S E K D I V H S G L A Y	496
122	Q H S Q Q H R T P C K G I R Y	136	1622	ACA ATG GAG AGA TCT GCC CGG CAA ATC ATG CGC ACA GCA AGC AAG	1666
542	AGC ACA GAA GTG TCT GTG GAT GAA GTT AAA GCC TTG GCC TCG CTG	586	497	T M E R S A R Q I M R T A S K	511
137	S T E V S V D E V K A L A S L	151	1667	TAC AAT CTG GGT CTG GAC CTG CGG ACG GCT GCT TAT GTT AAT GCC	1711
587	ATG ACC TAC AAG TGT GCT GTT GTG GAT VTG CCA TTT GGG GGA GCC	631	512	Y N L G L D L R T A A Y V N A	526
152	M T Y K C A V A V P F G G A	166	1712	ATC GAG AAA GTC TTC AAA GTC TAC AAT GAG GCG GAG CTC ACC TTC	1756
632	AAA GCT GGA GTC AAG ATC AAC CCC AAG AAT TAC TCT GAT AAC GAG	676	527	I E K V F K V Y N E A G L T F	541
167	K A G V K I N P K N Y S D N E	181		← CDS end	
677	CTG GAG AAG ATT ACC AGG AGG TTC ACT ATT GAA CTG GCC AAG AAG	721	1757	ACA TAA	
182	L E K I T R R F T I E L A K K	196	542	T *	1801
722	GGG TTC ATT GGA CCT GGC ATC GAT GTC CCG GCC CCA GAC ATG AGC	766	1802	ACC CTT AAA CAG CCT TCT CTT CAT ATC ACT GAT CAC CGC TGC AGT	1846
197	G F I G F G I D V P A F D M S	211	1847	TTA GCT CTT TCA CAC ATA TCC AGC GAG GCG CTG GCT AGC TTG GAC	1891
767	ACA GGA GAG AGG GAA ATG TCA TGG ATC GCT GAC ACG TAT GCC AAC	811	1892	TCT ACC CTG TGA GCT TTG TGC TGC CAT TAT ACT GAA TCT GCA TCT	1936
212	T G E R E M S W I A D T Y A N	226	1937	CTG TTT AAA TAT CGC TCT GTT CTT GTT TTT GCC TGA GGT TAT ATT	1981
812	ACC ATT GCA CAC ACT GAC ATC AAC GCC CAT GCA TGT GTG ACA GGA	856	1982	CAG AAG TAC CCC ATA GTG TAC CCT GAA AGC ACG TGA CCC TCT TGT	2026
227	T I A H T D I N A H A C V T G	241	2027	TGT AAC TTA CAG TTG ACA CCA CTA CAG ATG CCT TAA TTT TAG AAA	2071
857	AAA CCC ATC AGC CAG GGG GGT ATC CAT GGA CGT ATA TCA GCC ACT	901	2072	GAG GTC TGC TCT AAT AGG GTG ACT AAA CAC TTC AGT TTA ATT AAA	2116
242	K P I S Q G G I H G R I S A T	256	2117	GGG ATT TAG ATT TAA TTT TGT TAA TGA GAG GGG AAA TAA ATC ACT	2161
902	GGT CGT GGA GTT TTC CAC GGC GTC GAG AAC TTC ATT AAC GAG GCC	946	2162	GTT TTT GAG TTT GTC TTT TTA CTC TAA GGT TAC CAA TGA AGT TCC	2206
257	G R G V F H G V A E N F I N E A	271	2207	CAA GCT TTA GGA TTG TCA TAG TAT ATT TAC TGT TTT GCC TTC CCA	2251
947	TCC TAT ATG AGT TTG CTT GGC CTG AGC CCT GGC TTC CTA GAC AAA	991	2252	GTC CTT TAT CCT CGT TTA TCT CCT CCT AAC CGC AGC TTC TCC CTG	2296
272	S Y M S L L G L S P G F L D K	286	2297	ACT CCT CTG CTG GCC AAG AAA GGG GAG TGG GTG TCA CTA TGA CTG	2341
992	ACC TTC ATT GTG CAG GGC TTT GGT AAT GTG GGT CTC CAC TCC ATG	1036	2342	CCA GTC CCT TTT TCA GTT TTA TCC ATC CAG TTA CTC AAC ATT AAA	2386
287	T F I V Q G F G N V G L H S M	301	2387	ATA GTT TGA CTA AAT AAC TTT TTA TTT TGT TCA TTT ATG GAA ACC	2431
1037	AGG TAC CTC CAC AGG TTT GGG GCC AAG TGC GTG GGT ATT GGT GAA	1081	2432	AAT AAA GTG TTC AGA AAA AGC TAA AAA AAA AAA AAA AAA AAA	2476
302	R Y L H R F A V A K I G I G E	316	2477	AAA 2479	
1082	ATT GAT GGA GCC ATC TAC AAT CCA AAK GGC ATT GAC CCC AAA GAG	1126			
317	I D G A I Y N P E G I D P K Q	331			
1127	CTG GAG GAC TAC AAA CTG CAA TAT GGT ACC ATT GTG GGC TTC CCA	1171			
332	L E D Y K L Q Y G T I V G F P	346			

FIGURE 1 | The complete nucleotide sequence and the corresponding translated amino acid sequence of the complete coding sequence (CDS) of glutamate dehydrogenase 1a (*gdh1a*; GenBank accession no. JF694445) from the liver of *Monopterus albus*. "*" Indicates the stop

codon. The start and the end of the CDS are indicated in boldface type, and the priming positions of the RACE primers used are underlined and indicated in boldface type. Pentameric motifs corresponding to AU-rich elements (AREs) are highlighted in gray.

$\Delta\Delta C_t$ calculation for relative quantification of target gene with reference to actin was applied to obtain the fold change in gene expression. All data were normalized to the abundance of actin mRNA. Results were expressed in fold change but statistical analyses were performed after \log_2 transformation of the fold change values.

DETERMINATION OF Gdh ACTIVITY

Tissue samples ($N = 4$ for each organ and each condition) were homogenized three times in 5 volumes (w/v) of ice-cold extraction buffer containing 50 mmol l^{-1} imidazole-HCl (pH 7.0), 50 mmol l^{-1} sodium fluoride, 3 mmol l^{-1} EGTA, and 3 mmol l^{-1} EDTA at 24,000 r.p.m. for 20 s each with a 10-s off interval using an Ultra-Turrax homogenizer. The homogenates were centrifuged at $10,000 \times g$ and 4°C for 20 min. The supernatant obtained were passed through a 5-ml Econo-Pac 10DG desalting column (Bio-Rad Laboratories, Hercules, CA, USA) equilibrated with 50 mmol l^{-1} imidazole-HCl (pH 7.0) and eluted with the same buffer. The resulting eluents were used for the determination of Gdh activities. The dilution involved was corrected by monitoring the change in protein concentrations of the sample before and after passing through the column. Protein was determined according to the method of Bradford (1976). Bovine gamma globulin was used as a standard for comparison. The amination activity of GDH was determined according to Peng et al. (1994), and was expressed as micromole NADH oxidized per minute per gram tissue.

STATISTICAL ANALYSES

Results were presented as mean \pm SD. Student's t -test and one-way analysis of variance (ANOVA) followed by multiple comparison of means by the Tukey test were used to evaluate differences between means where applicable. Differences were regarded as statistically significant at $p < 0.05$.

RESULTS

gdh NUCLEOTIDE AND THE TRANSLATED Gdh SEQUENCES

At the beginning, partial fragments of *gdh* were obtained from the liver, intestine, and brain of control *M. albus* kept in freshwater or fish exposed to terrestrial conditions, environmental ammonia, or salinity stress through PCR using four pairs of *gdh* primers. There was no variation in the partial *gdh* sequences between tissues or between conditions. Furthermore, there was no sequence variation among the 5' RACE products or the 3' RACE products generated from the cDNA of the three organs. The complete *gdh* gene sequence (GenBank accession no.: JF694445) obtained from the liver of *M. albus* consisted of a 133-bp 5' UTR, a complete coding sequence (CDS) region spanning 1629 bp and a 3' UTR of approximately 717 bp (Figure 1). The same complete sequence was obtained for *gdh* from the intestine and brain of *M. albus*. In addition, there was no variation in the complete *gdh* sequences from all three tissues of fish exposed to terrestrial conditions, environmental ammonia, or salinity stress. The translated Gdh amino acid sequence obtained from the liver of *M. albus* had 542 residues (Figure 1), and a comparison with those of other animals revealed that it is closely related to Gdh1a (Table 2). From the alignment of the deduced *M. albus* Gdh1a

with Gdh from *C. aceratus*, *O. mykiss*, *T. hakonensis*, *X. laevis*, and *H. sapiens*, it is evident that Gdh is highly conserved across species (Figure 2).

PHYLOGENETIC ANALYSIS

Monopterus albus Gdh1a formed a monophyletic clade with *B. sinensis* Gdh1a, *T. nigroviridis* Gdh1a, *T. rubripes* Gdh1a, *C. aceratus* Gdh1a, *S. salar* Gdh1a1 and Gdh1a2, and *O. mykiss* Gdh1a (Figure 3). *Danio rerio* Gdh1a and Gdh1b, *T. hakonensis* Gdh1a, *B. sinensis* Gdh1b, *T. nigroviridis* Gdh1b, *S. salar* Gdh1b, and *O. mykiss* Gdh1b diverged from *M. albus* Gdh1a (Figure 3) and generally exhibited lower sequence identity scores (<90%) with *M. albus* Gdh1a.

EFFECTS OF VARIOUS ENVIRONMENTAL CONDITIONS ON THE mRNA EXPRESSION OF *gdh1a* IN THE LIVER, INTESTINE, AND BRAIN

The mRNA expression of *gdh1a* increased significantly by 2.6 and 3.2-fold in the liver of *M. albus* exposed to terrestrial conditions or 75 mmol l^{-1} NH_4Cl , respectively, for 1 day (Figure 4A), but it returned to the control level after 6 days of exposure to both conditions (Figure 4B). There was no significant change in the mRNA expression of *gdh1a* in the liver of fish exposed to brackish water for 1 day (Figure 4C). Exposure of fish to terrestrial conditions or 75 mmol l^{-1} NH_4Cl did not result in any significant changes in the mRNA expression of *gdh1a* in the intestine (Figures 5A,B). In contrast, there was a significant increase (2.5-fold) in the mRNA expression of *gdh1a* in the intestine of fish exposed to brackish water for 1 day (Figure 5C). On the other hand, the mRNA expression of *gdh1a* remained unchanged in the brain of *M. albus* exposed to all three experimental conditions (Figure 6).

Table 2 | Percentage similarity of GDH/Gdh from various organisms with *Monopterus albus* Gdh1a (GenBank accession no. JF694445) obtained using Cluster W multiple alignment.

	Similarity (%) with <i>M. albus</i> Gdh1a
<i>M. anguillicaudatus</i> Gdh1a	92
<i>O. mykiss</i> Gdh1a	93
<i>O. mykiss</i> Gdh1b	88
<i>T. nigroviridis</i> Gdh1a	94
<i>T. nigroviridis</i> Gdh1b	85
<i>D. rerio</i> Gdh1a	92
<i>D. rerio</i> Gdh1b	87
<i>S. salar</i> Gdh1a1	92
<i>S. salar</i> Gdh1a2	89
<i>S. salar</i> Gdh1b	88
<i>T. hakonensis</i> Gdh1a	92
<i>C. aceratus</i> Gdh1a	86
<i>B. sinensis</i> Gdh1a	96
<i>B. sinensis</i> Gdh1b	85
<i>L. vannamei</i> Gdh	68
<i>X. laevis</i> GDH1	88
<i>X. tropicalis</i> GDH1	87
<i>M. musculus</i> GDH1	84
<i>H. sapiens</i> GLUD1	84
<i>R. norvegicus</i> GDH1	84

<i>M. albus</i>	MYRYFGE--LLSRGASSALASGCVDSDALPL-----SSSLMRL	35	<i>M. albus</i>	LDKTFIVQGFNVLHSMRYLHRFGAKCVGIGEIDGAIYNPEGIDPKQLE	333
<i>C. aceratus</i>	-----	1	<i>C. aceratus</i>	QDKTFVIOGFGNVLHSMRYLHRFGAKCVGIGEIDGAIYNADGIDPKALE	295
<i>O. mykiss</i>	MYRYFGE--LLTRGASSALASGCVESALPV-----TASLMRV	35	<i>O. mykiss</i>	TDKTFVIOGFGNVLHSMRYLHRFGAKCVGEMDGNINWPNPIDPKLE	335
<i>T. hakonensis</i>	-----	1	<i>T. hakonensis</i>	ADKTFVIOGFGNVLHSMRYLHRFGAKCVGIEIDGSIWPNMGIDPKLE	281
<i>X. laevis</i>	MYRYIGE--LVSRGGG-ALASCTADSVLPL-----SAAPI-L	33	<i>X. laevis</i>	GDKTFVIOGFGNVLHSMRYLHRFGAKCVGIGEIDGTIWNPNPIDPKLE	331
<i>H. sapians</i>	MYRYLGEALLSRGAPALGSASADSAAALGWARGQPAAPQGLAALAA	50	<i>H. sapians</i>	GDKTFVIOGFGNVLHSMRYLHRFGAKCIAVSGSDSIWPNPIDPKLE	349
Consensus			Consensus	****:*****:*****:****:.* ** *:*:*** **	
<i>M. albus</i>	RHYADAAEQ--PDDPNFFKMVEGFFDRGASIVEDKLVEDLKTRESPEQKR	83	<i>M. albus</i>	DYKLYQGTIVGFPKAPYEGNILEADCHILIIPAAGEKQLTRNNAPRIKAK	383
<i>C. aceratus</i>	---ADAADK--PDDPNFFRMVGEFFDRGASIVEDKLVEDLRTKETPEQKK	45	<i>C. aceratus</i>	EYKLNQGTIVGFPKAPYEGSILEADCDILIIPAAGEKQLTRNNARRIKAK	345
<i>O. mykiss</i>	RHYSEVVEKDDADDPNFFKMVEGFFDRGANIVEDKLVEDLKNKTEPVQKR	85	<i>O. mykiss</i>	DYKLNQGTIVGFPNTPYEGSILEADCDILIIPAAGEKQLTRNNARRIKAK	385
<i>T. hakonensis</i>	-----MVGEFFDRGAAIVENKLVEDLKTRETPEQKR	31	<i>T. hakonensis</i>	EYKLNQGTIVGFPNTPYEGNILEADCDILIIPAAGEKQLTRNNARRIKAK	331
<i>X. laevis</i>	RRYSQAVNG--DDDPNFFKMVEGFFDRGASIVEDKLVEDLRTRETEEQKR	81	<i>X. laevis</i>	DYKLNQGTIVGFPKAPYDGNILEADCDILIIPAAGEKQLTKSNAHKIKAK	381
<i>H. sapians</i>	RHYSEAVADR--EDDPNFFKMVEGFFDRGASIVEDKLVEDLRTRESEEQKR	99	<i>H. sapians</i>	DFKLQHSILGFPKAPYEGSILEADCDILIIPAAGEKQLTKSNAPRVKAK	399
Consensus	***** **:*:****:.*:*		Consensus	:*** *:*:*** : **:*:***:*.*****:*****:*. * :***	
<i>M. albus</i>	NRVIRGILRIKPCNHVLSVSPFIKRDNGEWEVIEGYRAQHSQHRTPCKGG	133	<i>M. albus</i>	IIAEGANGPTTPADKIFLENNVMVDPMYLNAGGVTVSYFEWLKLNHV	433
<i>C. aceratus</i>	GRVAGILRIKPCNHVLSVSPFIKRDNGEWEVIEGYRAQHSQHRTPCKGG	95	<i>C. aceratus</i>	IIAEGANGPTTPADKIFLENNVMVDPMYLNAGGVTVSYFEWLKLNHV	395
<i>O. mykiss</i>	LRVIRGILRIKPCNHVLSVSPFIKRDNGEWEVIEGYRAQHSQHRTPCKGG	135	<i>O. mykiss</i>	IIAEGANGPTTPADKIFLENNVMVDPMYLNAGGVTVSYFEWLKLNHV	435
<i>T. hakonensis</i>	LRVIRGILRIKPCNHVLSVSPFIKRDNGEWEVIEGYRAQHSQHRTPCKGG	81	<i>T. hakonensis</i>	IIAEGANGPTTPADKIFLENNVMVDPMYLNAGGVTVSYFEWLKLNHV	431
<i>X. laevis</i>	LRVIRGILRIKPCNHVLSVSPFIKRDNGEWEVIEGYRAQHSQHRTPCKGG	131	<i>X. laevis</i>	IIAEGANGPTTPADKIFLENNVMVDPMYLNAGGVTVSYFEWLKLNHV	431
<i>H. sapians</i>	NRVIRGILRIKPCNHVLSVSPFIKRDNGEWEVIEGYRAQHSQHRTPCKGG	149	<i>H. sapians</i>	IIAEGANGPTTPADKIFLENNVMVDPMYLNAGGVTVSYFEWLKLNHV	449
Consensus	** **:*:*****:***:***:*. * :***:*****:*****:*****		Consensus	*****:*****:*. * :***:*****:*****:*****	
<i>M. albus</i>	IRYSTEVSDEVKALASLMTYKCAVVDVPPFGGAKAGVKINPNKNSDNELE	183	<i>M. albus</i>	SYGRLTFKYERDSNYHLLMSVQESLERKFKGKGGPIPIVPTADFOARVAG	483
<i>C. aceratus</i>	IRYSMDVSDEVKALASLMTYKCAVVDVPPFGGAKAGVINTPNKNSDNELE	145	<i>C. aceratus</i>	SYGRLTFKYERDSNYHLLMSVQESLERKFKGKGGPIPIVPTADFOARVAG	445
<i>O. mykiss</i>	IRYSTEVSDEVKALASLMTYKCAVVDVPPFGGAKAGVKINPNKNSDNELE	185	<i>O. mykiss</i>	SYGRLTFKYERDSNYHLLMSVQESLERKFKGKGGPIPIVPTADFOARVAG	485
<i>T. hakonensis</i>	IRYSMDVSDEVKALASLMTYKCAVVDVPPFGGAKAGVKINPNKNSDNELE	131	<i>T. hakonensis</i>	SYGRLTFKYERDSNYHLLMSVQESLERKFKGKGGPIPIVPTADFOARVAG	431
<i>X. laevis</i>	IRYSTEVSDEVKALASLMTYKCAVVDVPPFGGAKAGVKINPNKNSDNELE	181	<i>X. laevis</i>	SYGRLTFKYERDSNYHLLMSVQESLERKFKGKGGPIPIVPTADFOARVAG	481
<i>H. sapians</i>	IRYSDVSDEVKALASLMTYKCAVVDVPPFGGAKAGVKINPNKNSDNELE	199	<i>H. sapians</i>	SYGRLTFKYERDSNYHLLMSVQESLERKFKGKGGPIPIVPTADFOARVAG	499
Consensus	****:*****:*****:*****:***:***:*. * :***		Consensus	*****:*****:***:***:*. * :***:*****:*****:*****	
<i>M. albus</i>	KITRRFTIELAKKGFIPGIDVDPAPDMSTGEREMSWIADTYANTIAHTDI	233	<i>M. albus</i>	ASEKDIVHSLAYTMERSARQIMRTASKYNLGLDLRTAAYVNAIEKVFVK	533
<i>C. aceratus</i>	KITRRFTIELAKKGFIPGIDVDPAPDMSTGEREMSWIADTYANTIAHTDI	195	<i>C. aceratus</i>	ASEKDIVHSLAYTMERSARQIMRTASKYNLGLDLRTAAYVNAIEKVFVK	495
<i>O. mykiss</i>	KITRRFTIELAKKGFIPGIDVDPAPDMSTGEREMSWIADTYANTIAHTDI	235	<i>O. mykiss</i>	ASEKDIVHSLAYTMERSARQIMRTASKYNLGLDLRTAAYVNAIEKVFVK	535
<i>T. hakonensis</i>	KITRRFTIELAKKGFIPGIDVDPAPDMSTGEREMSWIADTYANTIAHTDI	181	<i>T. hakonensis</i>	ASEKDIVHSLAYTMERSARQIMRTASKYNLGLDLRTAAYVNAIEKVFVK	481
<i>X. laevis</i>	KITRRFTIELAKKGFIPGIDVDPAPDMSTGEREMSWIADTYANTIAHTDI	231	<i>X. laevis</i>	ASEKDIVHSLAYTMERSARQIMRTASKYNLGLDLRTAAYVNAIEKVFVK	531
<i>H. sapians</i>	KITRRFTIELAKKGFIPGIDVDPAPDMSTGEREMSWIADTYASTIGHYDI	249	<i>H. sapians</i>	ASEKDIVHSLAYTMERSARQIMRTASKYNLGLDLRTAAYVNAIEKVFVK	549
Consensus	*****:*****:*****:*****:*****:*. * :***		Consensus	*****:*****:*****:*****:*****:*. * :*****:*****	
<i>M. albus</i>	NAHACVTGKPISQGGIHGRISATGRGVFHGVIENFNEASYSMLLGLSPGF	283	<i>M. albus</i>	YNEAGLTFT	542
<i>C. aceratus</i>	NAHACVTGKPISQGGIHGRISATGRGVFHGVIENFNEASYSMLVGLTPGV	245	<i>C. aceratus</i>	YNEAGLTFT	504
<i>O. mykiss</i>	NAHACVTGKPISQGGIHGRISATGRGVFHGVIENFNEAAYSMLGLSPGF	285	<i>O. mykiss</i>	YNEAGLTFT	544
<i>T. hakonensis</i>	NAHACVTGKPISQGGIHGRISATGRGVFHGVIENFNEASYSMLGLTPGF	231	<i>T. hakonensis</i>	YNEAGLTFT	490
<i>X. laevis</i>	NAHACVTGKPISQGGIHGRISATGRGVFHGVIENFNEASYSMLGLMTPGF	281	<i>X. laevis</i>	YNEAGLTFT	540
<i>H. sapians</i>	NAHACVTGKPISQGGIHGRISATGRGVFHGVIENFNEASYSMLGLMTPGF	299	<i>H. sapians</i>	YNEAGTFT	558
Consensus	*****:*****:*****:*****:*****:***:***:*. * :***		Consensus	****:***	

FIGURE 2 | The alignment of the translated amino acid sequence of glutamate dehydrogenase 1a (Gdh1a) from the liver of *Monopterus albus* and the amino acid sequences of Gdh in *Chaenocephalus aceratus* (P82264.1), *Oncorhynchus mykiss* (NP_001166000.1), *Tribolodon hakonensis* (BAD83654.1), *Xenopus laevis* (NP_001087023.1), and *Homo sapiens* (NP_005262.1). Identical residues in the alignment are indicated by “*”; similar amino acids in the alignment are indicated by “.”; dissimilar amino acids in the alignment are indicated by “.”. Residues involved in adenine binding domain are boxed; residues contributing to the antenna domain are shaded gray.

sapiens (NP_005262.1). Identical residues in the alignment are indicated by “*”; similar amino acids in the alignment are indicated by “.”; dissimilar amino acids in the alignment are indicated by “.”. Residues involved in adenine binding domain are boxed; residues contributing to the antenna domain are shaded gray.

EFFECTS OF VARIOUS ENVIRONMENTAL CONDITIONS ON THE AMINATION ACTIVITY OF Gdh FROM THE LIVER, INTESTINE, AND BRAIN

There was a significant increase in the amination activity of Gdh from the liver of *M. albus* after 1 day of exposure to terrestrial conditions or 75 mmol l⁻¹ NH₄Cl, but the activity returned to the control level on day 6 (Table 3). One day or 6 days of exposure to terrestrial conditions or 75 mmol l⁻¹ NH₄Cl had no significant effect on the intestinal Gdh amination activity. As for the brain, there were no changes in the amination activity of Gdh except for fish exposed to 75 mmol l⁻¹ NH₄Cl for 6 days whereby the activity decreased significantly (Table 3). Exposure to brackish water had no significant effects on the amination activity of Gdh from the liver and brain of *M. albus*, but resulted in a significant increase (threefold) in the intestinal Gdh amination activity (Table 4).

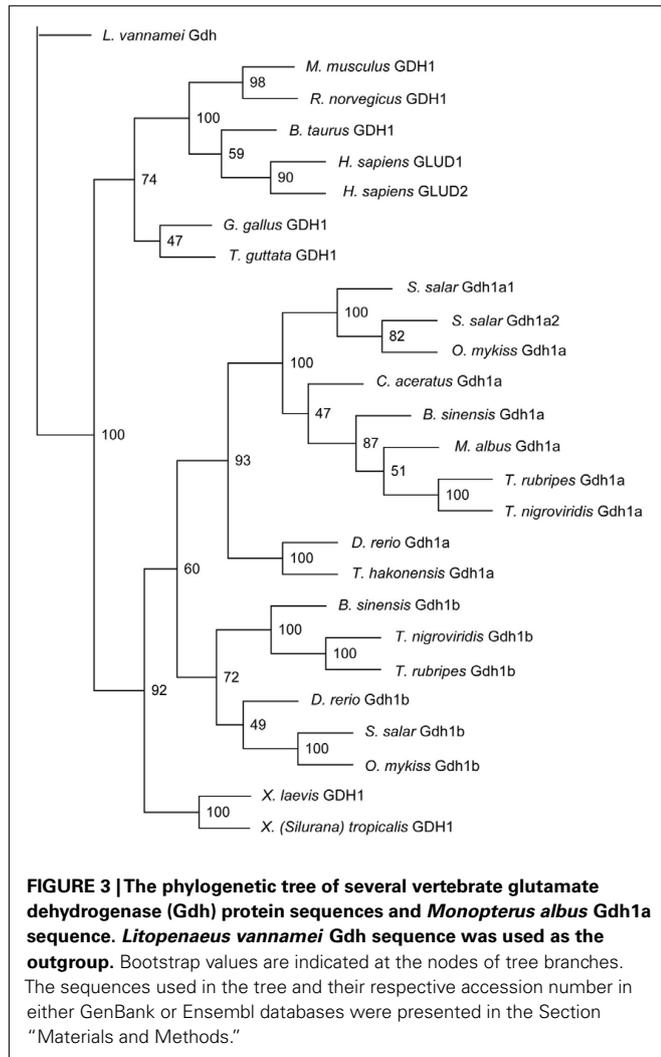
DISCUSSION

EXPRESSION OF Gdh1a IN THE LIVER, INTESTINE, AND BRAIN OF M. ALBUS

Two forms of GDH, *GLUD1* (ubiquitous), and *GLUD2* (nerve tissue specific) are expressed in human (Mavrothalassitis et al., 1988; Shashidharan et al., 1994, 1997). The hibernating Richardson's

ground squirrel also expresses two distinct forms of GDH (Thatcher and Storey, 2001). Similarly, two forms of *gdh* have been identified in *D. rerio* (Woods et al., 2005) and *B. sinensis* (Peh, 2008), and multiple isoforms of *gdh* in *O. mykiss* (AAM73775.1, AAM73776.1, AAM73777.1) and *S. salar* (CAD89353.1, CAD58714.1, CAD58715.1, CAD58716.1) can be found in GenBank. In contrast, only one form of *gdh* is expressed in the gills of *T. hakonensis*. In addition, the presence of only one form of Gdh in the liver of *C. aceratus* (Ciardiello et al., 2000) implies the expression of a single *gdh*. In this study, we identified only *gdh1a* from the liver, intestine, and brain of *M. albus*, but the possibility of the presence of other *gdh* isoforms, especially in other organs, cannot be ignored. The translated Gdh1a of *M. albus* had high sequence identity, and was grouped together, with those of *T. nigroviridis*, *T. Rubripes*, *B. sinensis*, *C. aceratus*, *S. salar*, and *O. mykiss*, and by coincidence, these fishes are capable of inhabiting waters of high salinities.

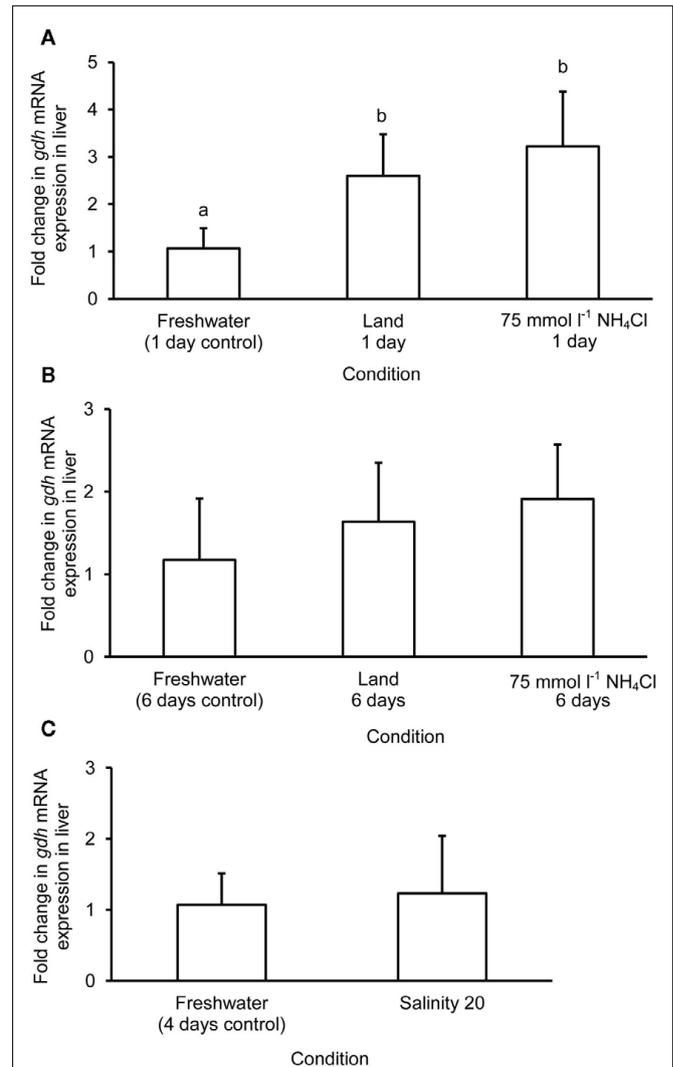
In general, the Gdh amino acid sequences of *M. albus* and other fishes, and those of amphibians and mammals are highly conserved. The activator of GDH, ADP, is noted to have varying stimulatory effects for different GDH proteins or isoforms (Cho et al., 1995; Ciardiello et al., 2000; Plaitakis and Zaganas, 2001;



Plaitakis et al., 2003). An adenine binding domain consisting of 14 consecutive amino acid residues exists within the ADP binding site in GDH, in which the Tyr187 residue is shown to be essential for GDH activation by ADP (Yoon et al., 2002). In *M. albus*, the amino acids contributing to the adenine binding domain and the antenna domain were identical to those in *T. hakonensis* and *C. aceratus*, but differed from that of *O. mykiss* Gdh1b and *X. laevis* GDH1. The GDH antenna domain, present only in GDH from multicellular animals, is apparently involved in allosteric regulation (Banerjee et al., 2003). Variations in the amino acid sequences in this region may confer selective advantages, allowing the GDH isoform to cater to the metabolic needs of an organism (Banerjee et al., 2003; Choi et al., 2007). Hence, it is probable that the allosteric regulation of Gdh1a in *M. albus* would bear some similarity with those in *T. hakonensis* and *C. aceratus*.

DIFFERENTIAL REGULATION OF *gdh1a* mRNA EXPRESSION IN THE LIVER, INTESTINE, AND BRAIN OF *M. ALBUS* EXPOSED TO TERRESTRIAL CONDITIONS OR ENVIRONMENTAL AMMONIA

The mRNA expression of *gdh1a* in the liver of *M. albus* exposed to terrestrial conditions or 75 mmol l⁻¹ NH₄Cl for 1 day increased



significantly, but returned to control levels on day 6. GDH is generally crucial to the regulation of amino acid catabolism and ammonia production. Excess amino acids from diet proteins are preferentially degraded, and their carbon skeletons can be channeled directly into the tricarboxylic acid cycle or converted to glucose through gluconeogenesis in the liver. The first step in amino acid catabolism in the liver involves the removal of the α -amino nitrogen as ammonia, and many amino acids are oxidatively deaminated by GDH after transamination (Campbell, 1991). Since *M. albus* were unfed during the experimental period, it is

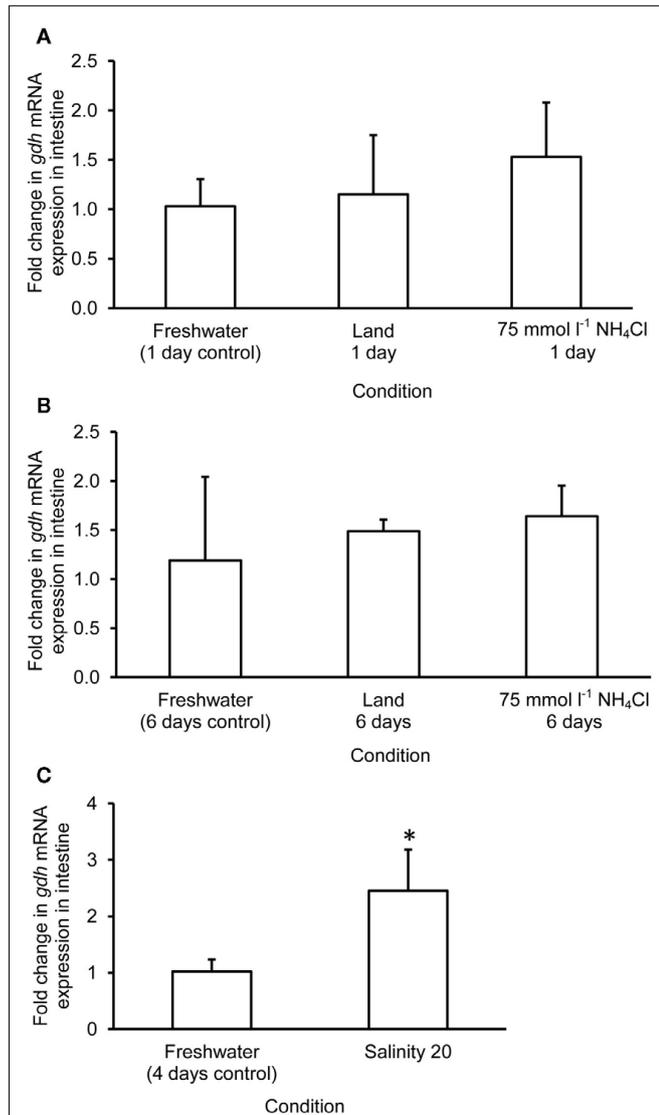


FIGURE 5 | Effects of (A) 1 day of exposure to freshwater (control), terrestrial conditions, or 75 mmol l⁻¹ NH₄Cl, (B) 6 days of exposure to freshwater (control), terrestrial conditions (land), or 75 mmol l⁻¹ NH₄Cl, and (C) 4 days of exposure to freshwater (control) or 1 day of exposure to salinity 20 after a 3-day progressive increase in salinity from freshwater on the mRNA expression (fold change) of glutamate dehydrogenase 1a (*gdh1a*) in the intestine of *Monopterus albus*.

Results represent mean + SD ($N = 4$). Statistical analysis was applied after \log_2 transformation of the individual fold change values. *Significantly different from corresponding control, $p < 0.05$.

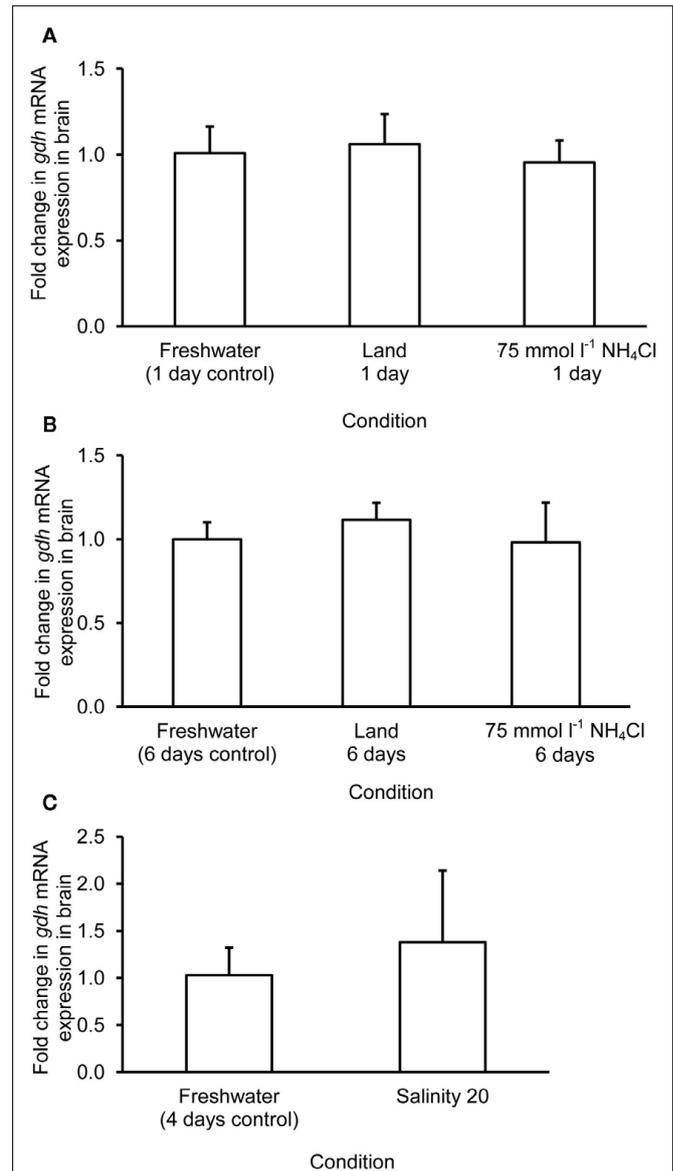


FIGURE 6 | Effects of (A) 1 day of exposure to freshwater (control), terrestrial conditions, or 75 mmol l⁻¹ NH₄Cl, (B) 6 days of exposure to freshwater (control), terrestrial conditions (land), or 75 mmol l⁻¹ NH₄Cl, and (C) 4 days of exposure to freshwater (control) or 1 day of exposure to salinity 20 after a 3-day progressive increase in salinity from freshwater on the mRNA expression (fold change) of glutamate dehydrogenase 1a (*gdh1a*) in the brain of *Monopterus albus*.

Results represent mean + SD ($N = 4$). Statistical analysis was applied after \log_2 transformation of the individual fold change values.

unlikely that the initial increase in *gdh1a* mRNA expression was to facilitate glutamate catabolism. In fact, it has been established that *M. albus* suppresses amino acid catabolism and detoxifies ammonia to glutamine during terrestrial and ammonia exposure (Tay et al., 2003; Ip et al., 2004c). Hence, the initial increase in *gdh1a* mRNA expression could be a response to increase glutamate production to support the increased synthesis of glutamine catalyzed by GS. It has been demonstrated in mammals that hepatic *GDH* mRNA expression is up-regulated in the pericentral zones,

resulting in increases in *GDH* activity and consequently glutamate production in support of the increased detoxification of ammonia to glutamine after feeding (Boon et al., 1999). In fish, such as the scale-less carp *Gymnocypris przewalskii* (Wang et al., 2003) and the air-breathing catfish *Clarias batrachus* (Saha et al., 2000), hepatic *Gdh* activities are up-regulated following exposure to ammonia. Thus, the initial up-regulation of hepatic *gdh1a* mRNA expression in the liver of *M. albus* exposed to terrestrial conditions or

Table 3 | The amination activity ($\mu\text{mol NADH oxidized min}^{-1} \text{g}^{-1}$ tissue) of glutamate dehydrogenase from the liver, intestine, and brain of *Monopterus albus* kept in freshwater (control) or after 1 day or 6 days of exposure to terrestrial conditions or 75 mmol l^{-1} NH_4Cl .

	Freshwater (control)	Terrestrial conditions		75 mmol l^{-1} NH_4Cl	
		1 day	6 days	1 day	6 days
Liver	35.4 \pm 4.6 ^a	45.8 \pm 3.3 ^b	33.7 \pm 5.9 ^a	49.1 \pm 4.4 ^b	29.7 \pm 3.6 ^a
Intestine	2.33 \pm 0.43	2.14 \pm 0.41	2.27 \pm 0.34	1.96 \pm 0.56	2.38 \pm 0.31
Brain	25.7 \pm 2.3 ^b	22.1 \pm 3.4 ^b	24.8 \pm 2.7 ^b	21.4 \pm 2.1 ^b	16.2 \pm 1.8 ^a

Results represent mean \pm SD (N = 4).

Means not sharing the same letter are significantly different, $p < 0.05$.

ammonia could be an adaptation to increase glutamate synthesis in preparation of the detoxification of ammonia to glutamine.

Tay et al. (2003) and Ip et al. (2004c) reported that there were significant changes in the Gdh activity in the liver of *M. albus* after 6 days of exposure to terrestrial conditions and environmental ammonia, respectively, but they did not examine effects of a shorter period of exposure to these conditions. Results obtained from this study confirmed that an increase in Gdh amination activity occurred in the liver of *M. albus* after 1 day of exposure to terrestrial conditions or environmental ammonia. However, both hepatic *gdh1a* mRNA expression and hepatic Gdh amination activity returned to control level on day 6. Post-transcriptional control through the regulation of mRNA stability and translation are important in mediating gene expressions (Ross, 1995; Mitchell and Tollervy, 2000, 2001; Guhaniyogi and Brewer, 2001). An AU-rich element (ARE) is defined as a region with frequent adenine and uridine bases in the 3' UTR of mRNA. It usually targets the mRNA for degradation (Chen and Shyu, 1995; Wilson and Brewer, 1999; Barreau et al., 2005), although there are a few instances where ARE-binding proteins may stabilize the mRNA transcript (Schroeder et al., 2003; Barreau et al., 2005). Present in the 3' UTR of *gdh1a* from *M. albus* are random ATTTA pentamers (Figure 1) that corresponds to AUUUA pentamers, a type of Class I AREs (Chen and Shyu, 1995). The existence of these AREs in *M. albus* suggested that increases in *gdh1a* mRNA transcripts in response to 1 day of exposure to terrestrial conditions or 75 mmol l^{-1} NH_4Cl were probably transient, corroborating the observation that the mRNA expression of *gdh1a* mRNA returned to control level on day 6.

As for the intestine of *M. albus*, the mRNA expression of *gdh1a* and Gdh amination activity remained unchanged after 1 day or 6 days of exposure to terrestrial conditions or 75 mmol l^{-1} NH_4Cl , which is in agreement with previous reports that 6 days of emersion or ammonia exposure have no significant effect on the intestinal Gdh activity in *M. albus* (Tay et al., 2003; Ip et al., 2004c). By contrast, Peh et al. (2010) reported that there were significant increases in the ammonia content, and amination and deamination activities and protein abundance of Gdh, in the intestine of *B. sinensis* exposed to 15 mmol l^{-1} NH_4Cl in seawater (pH 7.0). The excess glutamate formed in the intestine was apparently channeled into other amino acids and/or transported to other organs. However, when *B. sinensis* was exposed to a much higher concentration (30 mmol l^{-1}) of NH_4Cl in water at salinity 50 (pH. 7.0), the magnitude of increase in ammonia content in the intestine was less prominent, and there were no changes in

Table 4 | The amination activity ($\mu\text{mol NADH oxidized min}^{-1} \text{g}^{-1}$ tissue) of glutamate dehydrogenase from the liver, intestine, and brain of *Monopterus albus* kept in freshwater (control) or in water at salinity 20 for 1 day after a 3-day progressive increase in salinity from freshwater.

	Freshwater	Salinity 20
Liver	37.5 \pm 5.9	29.9 \pm 7.5
Intestine	2.15 \pm 0.89	6.30 \pm 1.53*
Brain	23.3 \pm 3.6	26.2 \pm 4.7

Results represent mean \pm SD (N = 4).

*Significantly different from the freshwater control, $p < 0.05$.

activities and kinetic properties of intestinal Gdh. Therefore, Peh et al. (2010) proposed that intestinal Gdh could be involved in the defense against ammonia toxicity during exposure to ammonia in a hyperosmotic medium, whereby drinking was essential for osmoregulation. Since *M. albus* would refrain from imbibing water in a freshwater environment, the lack of effects of ammonia exposure on the mRNA expression of *gdh1a* and on the Gdh amination activity in its intestine is in support of the proposition of Peh et al. (2010).

One day or 6 days of terrestrial or ammonia exposure had no significant effects on the mRNA expression of *gdh1a* in the brain of *M. albus*. Indeed, there was no change in the Gdh amination activity from the brain of *M. albus* exposed to terrestrial conditions. However, in agreement with the report of Ip et al. (2004c), our results indicated that 6 days of exposure to ammonia led to a significant decrease in brain Gdh activity in *M. albus*. Hence, it is logical to deduce that a change in the protein expression of Gdh at the translation level could have occurred in the brain of fish after 6 days of exposure to 75 mmol l^{-1} NH_4Cl .

UP-REGULATION OF *gdh1a* mRNA EXPRESSION IN THE INTESTINE OF *M. ALBUS* EXPOSED TO BRACKISH WATER

It has been reported recently that *M. albus* exposed to brackish water accumulates glutamine as a major organic osmolyte for cell volume regulation (Tok et al., 2009). The activity and protein abundance of GS increase significantly in the liver and muscle of fish exposed to brackish water. Increased production of glutamine requires an increased supply of glutamate, and indeed our results indicated that a significant increase in *gdh1a* mRNA

expression occurred in the intestine of *M. albus* exposed to brackish water, which corroborates the findings of increased intestinal Gdh amination activity in this and previous (Tok et al., 2009) studies. Since the juvenile marble goby, *Oxyeleotris marmorata*, also up-regulates intestinal Gdh activity and protein abundance during seawater acclimation (Chew et al., 2010), it can be concluded that the intestines of some euryhaline fishes have an important osmoregulatory function of upregulating glutamate synthesis in response to salinity stress. The excess glutamate could be transported to other organs in support of increased glutamine synthesis for cell volume regulation.

CONCLUSION

The liver, intestine, and brain of *M. albus* expressed *gdh1a*, the translated amino acid of which had the greatest sequence

identity with *O. mykiss* Gdh1a, *S. salar* Gdh1a1, *B. sinensis* Gdh1a, and *T. hakonensis* Gdh1a. The mRNA expression of *gdh1a* was differentially up-regulated in the liver and intestine of *M. albus* in response to ammonia toxicity (terrestrial conditions or environmental ammonia) and salinity stress (brackish water), respectively. It is probable that the corresponding increases in Gdh amination activities in these two organs would lead to increases in the production of glutamate to support increased glutamine synthesis for ammonia detoxification or cell volume regulation.

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