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Adaptive Changes in the Glucose Metabolism of a Bivalve to Ambient Ammonia Stress

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Representatives of the class bivalvia are very important for evaluating the level of the pollution of given area because the group comprises sedentary filter feeders which can accumulate xenobiotics from the environment. They are suspension feeders in the primary stages of food chain and influence the organization and functioning of the ecosystem (Mane et al. 1986). Mussels accumulate heavy metals like copper, zinc, iron, and lead (Hunter et al. 1987; King and Davies 1987). Global mussel policy established in 1978, international mussel watch programme in India (1986), and satellite symposium in Bhopal (1986) on biomonitors and biopurifiers highlighted the role of bivalves in biopurification, bioindication, and biomonitoring. Ammonia has received increased attention over the past few years as potentially important pollutant in aquatic systems. Animal feed lots, oil shale retorting, and coal gasification are some of the sources for ammonia pollution in aquatic ecosystems. The nitrogen fertilizers including ammonium sulfate which are not completely used up by the crops washed into nearby ponds and lakes through streams contributing ammonia toxicity to aquatic fauna. Information pertaining to the effects of ammonium salts on various animals is well documented (Ram and Singh 1988; Subhadra and Bhattacharya 1989). The present study was undertaken to investigate the effect of ammonia on glucose utilization by freshwater mussel (Chetty and Indira (in press)).

MATERIALS AND METHODS

The freshwater mussels, belonging to the species Lamellidens marginalis weighing 30 ± 2 g were collected from the ponds of neighboring village Karakambadi. Animals were washed under running tap water and maintained in large cement aquaria under 12:12 light: dark period. The physicochemical characteristics of tap water in which animals were maintained are as follows: temperature 26 ± 2°C; pH 7.0 ± 0.2; hardness, 61 mg/L (as HCO_3^-) and dissolved oxygen, 5.38 ± 0.72 mL/L. The animals were fed ad libitum on freshwater plankton and acclimated to laboratory conditions for a period of 15 d.

The toxicity tests were conducted using static water bioassay as suggested by Doudoroff et al. (1951). Batches of 10 animals were

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exposed to different concentrations of ammonium sulfate (purchased from E.Merk, India Limited; assay as dried substance -99%) ranging from 1000 to 5000 mg/L with an interval of 100 mg. After 48 hr the number of animals that died at each concentration was recorded. The animal was considered dead when the mantle edge was no longer responsive to touch and the animal failed to close the shell valves. Each experiment in the selected concentrations of ammonium sulfate was repeated six times. The mortality in each concentration was taken to determine the LC_{50} (3528 mg/L) by graphical plots of percent mortality, probit mortality against log concentration of ammonium sulfate (Finney 1964). Two concentrations of ammonium sulfate, 10 mg/L (which approximates the ammonia concentration in natural pond water) and 176 mg/L (sublethal concentration) were selected and six mussels were exposed to above two concentrations separately for 7 d. The control animals were maintained under identical conditions without ammonium sulfate in the medium. After the stipulated period, mantle, gill, foot, and hepatopancreas were excised under cold conditions (4°C) and frozen immediately in liquid nitrogen and stored at -80°C for biochemical analysis.

The total carbohydrate content was estimated by the method of Carrol et al. (1956) using trichloroacetic acid as homogenizing medium. The color developed by anthrone reagent was read at 620 nm in a spectrophotometer against the reagent blank. For the estimation of glucose (Mendel et al. 1954) and glycogen (Kemp and Hejnigen 1954), 80% methanol was used as extracting medium. Supernatant and residue were used for the estimation of glucose and glycogen respectively. The color developed with concentrated sulphuric acid was read at 520 nm in a spectrophotometer against the reagent blank. Glycogen phosphorylase activity in the tissues was assayed by the method of Cori et al. (1955) in the direction of glycogen synthesis by estimating the amount of inorganic phosphate formed from glucose-1-phosphate using amino naphthol sulfonic acid (ANSA) reagent. Aldolase activity was assayed by the colorimetric determination of triosephosphates formed with 2,4-dinitrophenyl hydrazine following the method of Bruns and Bergmeyer (1965). Glucose-6-phosphate dehydrogenase (Lohr and Waller 1965) and lactate dehydrogenase (Srikanthan and Krishnamoorthy 1955) were assayed using sucrose as homogenizing medium. For these enzyme assays (2-p-idophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) was used.The enzyme assays were made after due standardization of Optimal conditions. Statistical significance of the difference between control and experimental values was calculated using student's 't' test.

RESULTS AND DISCUSSION

The changes in glucose metabolism in four tissues of freshwater mussel exposed to two different concentrations of ambient ammonia are presented in Tables 1 and 2. The decrease in carbohydrates under ammonia stress (Tables 1 and 2) indicates their possible utilization to meet higher energy needs warranted by ammonia toxicity. Greater depletion in total carbohydrates at 176 mg/L exposure than at 10 mg/L suggests that the carbohydrate utilization depends on the concentration of ambient ammonium sulfate. Drop in glycogen content

Table 1. Changes in glucose metabolism of mantle and foot of freshwater mussel under exposure to 10 or 176 mg/L ammonium sulfate stress for 7 d.

Name of the Parameter	$(NH_4)_2 SO_4$ concentration, mg/L					
	Mantle			Foot		
	0	10	176	0	10	176
Total carbohydrates (mg glucose/g wet weight of the tissue)	54.59 ±3.086	49.07 ±3.413 (-10)	36.20 ±2.00 (-34)	19.47 ±1.214	12.88 ±0.461 (-34)	9.31 ±0.73 (-52)
Glycogen (mg glucose/g wet wieght of the tissue)	8.222 ±0.261	7.379 ±0.552 (-16)	4.972 ±0.217 (-44)	2.240 ±0.139	1.871 ±0.107 (-17)	1.143 ±0.218 (-49)
Glucose (mg glucose/g wet weight of the tissue)	0.360 ±0.017	0.290 ±0.014 (-19)	0.652 ±0.047 (+81)	0.154 ±0.011	0.113 ±0.010 (-27)	0.258 ±0.024 (+68)
Phosphorylase "a" (µmoles of pi formed/ mg/protein/hr)	0.709 ±0.02	0.913 ±0.073 (+29)	1.112 ±0.043 (+57)	0.854 ±0.032	0.974 ±0.2 (+14)NS	1.25 ±0.09 (+46)
Phosphorylase "b" (µmoles of pi formed/ mg/protein/hr)	0.324 ±0.038	0.308 ±0.023 (-6.1)NS	0.205 ±0.019 (-38)	0.326 ±0.053	0.354 ±0.026 (+8.6)NS	0.206 ±0.063 (-37)
Aldolase (µmoles of fructose 1,6-diphosphate cleaved/mg/protein/hr)	0.974 ±0.05	1.481 ±0.059 (+52)	1.869 ±0.091 (+92)	1.644 ±0.150	2.194 ±0.112 (+34)	3.936 ±0.202 (+139)
Lactate dehydrogenase (µmoles of formazan formed/ mg/protein/hr)	0.267 ±0.001	0.156 ±0.010 (-42)	0.099 ±0.007 (-63)	0.116 ±0.002	0.138 ±0.008 (+19)	0.093 ±0.010 (+37)
Glucose-6-phosphate dehydrogenase (µmoles of formazan formed/mg/protein/hr)	0.195 ±0.007	0.231 ±0.007 (+19)	0.278 ±0.012 (+43)	0.485 ±0.015	0.560 ±0.0.28 (+16)	0.348 ±0.014 (-28)

All the values are mean ± SD of 6 different specimens. Values in parantheses are percent changes over controls.
All the experimental values are significantly different from controls at $P < 0.001$ except NS = Not significant.

Table 2. Changes in glucose metabolism of gill and hepatopancreas of freshwater mussel under exposure to 10 or 176 mg/L ammonium sulfate stress for 7 d.

Name of the Parameter	$(NH_4)_2 SO_4$ concentration, mg/L					
	Gill			Hepatopancreas		
	0	10	176	0	10	176
Total carbohydrates (mg glucose/g wet weight of the tissue)	20.24 ±1.539	15.64 ±0.697 (-23)	10.20 ±0.633 (-50)	48.94 ±2.395	38.27 ±1.745 (-22)	18.49 ±1.155 (-62)
Glycogen (mg glucose/g wet wieght of the tissue)	2.782 ±0.113	3.961 ±0.218 (+42)	2.146 ±0.113 (-23)	5.595 ±0.268	4.055 ±0.216 (-28)	1.463 ±0.243 (-74)
Glucose (mg glucose/g wet weight of the tissue)	0.219 ±0.014	0.111 ±0.015 (-49)	0.408 ±0.022 (+86)	2.059 ±0.083	1.019 ±0.070 (-51)	1.509 ±0.101 (-27)
Phosphorylase "a" (µmoles of pi formed/ mg/protein/hr)	1.118 ±0.004	0.911 ±0.032 (-19)	1.395 ±0.032 (+25)	1.466 ±0.047	2.087 ±0.201 (+42)	2.571 ±0.1 (+75)
Phosphorylase "b" (µmoles of pi formed/ mg/protein/hr)	0.807 ±0.041	0.562 ±0.073 (-30)	0.656 ±0.012 (-19)	0.784 ±0.107	0.606 ±0.158 (-23)*	0.512 ±0.035 (-35)
Aldolase (µmoles of fructose 1,6-diphosphate cleaved/mg/protein/hr)	1.052 ±0.028	0.740 ±0.055 (-30)	1.316 ±0.077 (+25)	0.506 ±0.053	0.573 ±0.025 (+13)*	0.714 ±0.036 (+41)
Lactate dehydrogenase (µmoles of formazan formed/ mg/protein/hr)	0.274 ±0.017	0.129 ±0.011 (-52)	0.087 ±0.012 (-68)	0.258 ±0.029	0.148 ±0.009 (-43)	0.067 ±0.036 (-73)
Glucose-6-phosphate dehydrogenase (µmoles of formazan formed/mg/protein/hr)	0.223 ±0.015	0.257 ±0.015 (+15)	0.132 ±0.017 (-41)	0.205 ±0.008	0.240 ±0.026 (+17)*	0.221 ±0.018 (+7.8)**

All the values are mean ± SD of 6 different specimens. Values in parantheses are percent changes over controls.
All the experimental values are significantly different from controls at $P < 0.001$ except where indicated.
* Significant $P < 0.01$, ** Significant $P < 0.05$. NS = Not significant.

was more in the tissues at 176 mg/L concentration than at 10 mg/L except in gill. Decrease in glycogen content might be attributed to the mobilization of the energy substrate through glycolysis, since both aldolase and phosphorylase "a" activities were significantly elevated in all the tissues at 176 mg/L.

At 10 mg/L concentration all the tissues showed decrease in glucose levels. But there is significant increase of glucose in all the tissues at 176 mg/L concentration except hepatopancreas (Tables 1 and 2), since the hepatopancreas is involved in glycogen breakdown into glucose moieties and their transport to other tissues to meet the energy crisis during ammonia stress. The increase in glucose levels at 176 mg/L exposure in mantle, gill, and foot suggest either rapid turnover of these energy fuels by transportation from hepatopancreas or their lesser utilization for energy formation. Glucose utilization through hexosemonophosphate shunt (HMP) was accelerated as evinced by elevated glucose-6-phosphate dehydrogenase activity in all the tissues at 10 mg/L and in mantle and hepatopancreas at 176 mg/L. However the decrease in glucose-6-phosphate dehydrogenase and increase in aldolase activity in gill and foot at 176 mg/L concentration suggest that the glucose utilization in these two tissues was through anaerobic glycolysis. Similar trend was observed during pesticide stress in Lamellidens marginalis (Swami et al. 1983).

The statistically significant increase in phosphorylase "a" activity (Tables 1 and 2) in the tissues of mussels at 176 mg/L confirms the active breakdown of tissue glycogen for metabolic processes to meet augmented energy demand under stress conditions. Rapid conversion of the inactive "b" form into "a" form elevates phosphorylase "a" activity with a consequent decrease in the phosphorylase "b" activity in the tissues of experimental mussels under toxic stress (Tables 1 and 2).

Aldolase activity levels were found to increase significantly (Tables 1 and 2) in the tissues of ammonia treated freshwater mussels at both the concentrations except gill at 10 mg/L. In both the concentrations foot and mantle showed maximum elevation in enzyme activity indicating active conversion of hexoses to trioses. Since these two muscular tissues need high energy, these have greater potential to operate glycolysis at higher rates to overcome ammonia toxicity, the toxic effects are being countered to large extent in these two tissues as compared with gill and hepatopancreas.

Decreased glycogen levels and elevated phosphorylase "a" and aldolase activity in the tissues of mussels under ammonia stress indicated active operation of glycolytic pathway. On exposure to 10 mg/L and 176 mg/L concentrations of ammonium sulfate, oxidation of lactate is decreased in all the tissues, except in foot at 10 mg/L concentration. The decreased LDH activity suggests that conversion of lactate to pyruvate is not favored. In agreement with these studies increased lactate and decreased LDH were observed in freshwater mussel, Lamellidens marginalis, under carbon tetrachloride (Santhakumari 1990) stress condition.

Activity levels of G-6-PDH as shown in Tables 1 and 2 increased in

all the tissues of ammonium sulfate exposed animals except gill and foot at 176 mg/L concentration. The elevated enzyme activity in the experimental tissues indicates increased oxidation of glucose through HMP shunt in view of impaired oxidative metabolism to overcome the energy crisis caused by ammonia. Decreased G-6-PDH activity in gill and foot at 176 mg/L concentration might be due to glucose oxidation through glycolysis rather than HMP pathway. Kohli et al. (1975) suggested that NADPH plays a key role in the detoxification of toxic substances. In the present study, the NADPH generated through HMP shunt might have been used primarily for the detoxification of ammonia. Thus increased G-6-PDH might appear as a metabolic adaptation to protect the animal from ammonia toxicity and to increase the survival chances of the animal in water with elevated ammonia.

The present findings demonstrate that the ammonium sulfate exposure causes a significant shift in glucose metabolism from aerobic to anaerobic and pentosephosphate shunt in mantle and hepatopancreas to mitigate ammonia toxicity.

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