Enzyme Activities of Gill, Hepatopancreas, Mantle, and Adductor Muscle of the Oyster (*Crassostrea virginica*) after Changes in Diet and Salinity

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We examined the effects of exposure to low salinity (1/3 seawater) on the maximal activities of enzymes of lipid oxidation, amino acid and ketone body metabolism, gluconeogenesis, and glycolysis in gill, mantle, hepatopancreas, and adductor muscle of the oyster (*Crassostrea virginica*). No significant metabolic reorganization occurred after 4–6 wk in low salinity. The few significant differences which were found between enzyme activities of high- and low-salinity treatment groups were always due to lower activities in the low-salinity group. In particular, hexokinase, fructose bisphosphatase, and 3-hydroxyacyl CoA dehydrogenase declined in gill, hepatopancreas, and adductor muscle. Since high ionic strength depresses the activity of many enzymes, the reduced ionic strength in the cells of the low-salinity group may perturb metabolism by elevating rates of enzyme activity. As indicated by the present study, part of the response to low salinity by euryhaline molluscs may involve reducing the activity of certain enzymes to achieve a homeostasis of metabolic function.

On a étudié les effets de la salinité réduite (1/3 d'eau salée) sur l'activité maximale des enzymes de l'oxydation lipidique, du métabolisme des acides aminés et des corps cétoniques, de la gluconéogenèse et de la glycolyse dans les branchies, le manteau, l'hépato-pancréas et le muscle adducteur de l'huître (*Crassostrea virginica*). Aucune réorganisation métabolique n'était notable au bout de 4 à 6 sem en salinité réduite. Les quelques différences significatives observées entre les groupes gardés à salinité élevée et réduite étaient toujours dues à des activités enzymatiques moindres chez le groupe gardé à salinité réduite. Plus particulièrement, on a noté la baisse de l'activité de l'hexokinase, de la fructose-biphosphatase et de la 3-hydroxyacyl-CoA-déshydrogénase dans les branchies, l'hépatopancréas et le muscle adducteur. Étant donné qu'à concentration ionique élevée l'activité de nombreuses enzymes diminue, le fait que la concentration ionique soit réduite dans les cellules des huîtres gardées à salinité réduite peut perturber le métabolisme en stimulant l'activité enzymatique. Comme on l'a constaté dans l'étude présentée ici, en partie, la réaction des mollusques euryhalins à une baisse de salinité peut être une diminution de l'activité de certaines enzymes pour le maintien de l'homéostase de la fonction métabolique.

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Changes in extra- (Hand and Stickle 1977) and intracellular inorganic ion levels (Bricteux-Gregoire et al. 1964) occur in response to changing external salinities in many osmoconforming marine invertebrates. Such changes may have substantial effects on the metabolism of these organisms. Recent investigations of the oxidative properties of mitochondria of marine bivalves indicate a stimulation of oxidation of substrates from a variety of metabolic pathways in response to an abrupt shift to low osmolarities. Oxidation of pyruvate (Ballantyne and Moyes 1987a), some but not all amino acids (Ballantyne and Storey 1983, 1985; Ballantyne and Moon 1985; Ballantyne and Moyes 1987b, 1987c; Moyes and Ballantyne 1987), and some chain lengths of acyl carnitines (Ballantyne and Storey 1984; Ballantyne and Moyes 1987a) is enhanced at low salinities.

Some of the increases in respiration found in isolated mitochondria under hypoosmotic conditions are likely due to increased enzyme activity at low ionic strength. Investigations of the effects of acute hypoosmotic stress on the oxidation of glutamate in oyster gill mitochondria indicate that changing ionic strength in the mitochondrial matrix stimulates the electron transport chain resulting in elevated oxidation (Ballantyne and Moyes 1987c). Numerous metabolic enzymes are influenced by changing ionic strength (Chaplin et al. 1965; Gilles 1969; Wood 1973; Sarkissian 1974; Sugden and Newsholme 1975). The effects of changes in intracellular ionic strength as occur during salinity acclimation in euryhaline molluscs may disrupt metabolism by affecting enzyme activity. To maintain metabolic function in the face of lower intracellular ionic strength, adjustments in the levels of some metabolic enzymes may occur with long-term acclimation to hypoosmotic stress (Gilles 1969; Ballantyne et al. 1987).

Long-term metabolic responses to changing salinity have not been investigated in pathways other than those involving amino

acids. The present study was undertaken to determine the effects of exposure to low salinity on the activities of enzymes of various metabolic pathways in tissues of the oyster (*Crassostrea virginica*). In addition, to assess changes occurring in the metabolism of the oysters during the treatment period due to holding conditions or diet, the levels of enzymes were measured at the beginning and the end of the treatment period.

Materials and Methods

Animals

Malpeque oysters were obtained from a local seafood supplier and maintained at the Marine Biology Laboratory at the University of Guelph in seawater (1000 mosmol/L) for 2 wk before beginning the experiments. The animals were obtained in June and initial measurements were made within 1 wk. The other measurements were made in August after the treatment period. Each week throughout the study the oysters were fed algae and emulsions of herring. The temperature was 10 ± 1°C throughout the study. Animals were transferred directly to 1/3-strength seawater or retained in full-strength seawater for 4–6 wk before enzymes were measured. Ammonium ion levels in the water were monitored weekly and did not rise above 0.2 mg/L during the study.

Tissue Preparation

Tissues were removed from animals immediately after removal from the water and placed in ice-cold buffer (50 mM imidazole, pH 7.4, at 20°C). In the case of hydroxymethylglutaryl-CoA lyase, 3-oxoacid transferase, acetoacetyl-CoA thiolase, carnitine palmitoyl transferase, and carnitine octanoyl transferase, 0.2% triton X-100 was added to the homogenization buffer. The tissue was homogenized with a Polytron tissue homogenizer using three 10-s bursts. The homogenizer was set at a submaximal speed (five of nine possible settings). The homogenate was centrifuged at 18 200 × *g* for 20 min. The resulting supernatant was used in subsequent assays.

Enzyme Measurements

All enzyme measurements were carried out in duplicate at 10°C. Substrate concentrations and amounts of coupling enzyme were optimized with tissues from full-strength seawater animals. Hepatopancreas enzymes were always measured first and citrate synthase and carnitine octanoyl transferase and carnitine palmitoyl transferase were measured first in all tissues. NADH was prepared every 4 h and all other substrates were prepared fresh daily. Conditions for each assay are outlined below.

Hexokinase (HK) (EC[1] 2.7.1.1): 50 mM imidazole, pH 7.1, at 20°C, 1 mM glucose, 5 mM $MgCl_2$, 1 mM ATP, 0.16 mM NADP, 2 units glucose-6-phosphate dehydrogenase (G6PDH).

Pyruvate kinase (PK) (EC 2.7.1.40): 50 mM imidazole, pH 7.1, at 20°C, 5 mM ADP, 10 mM $MgCl_2$, 0.2 mM NADH, 50 mM KCl, 5.0 mM phosphoenolpyruvate, 1 unit lactate dehydrogenase (LDH).

Fructose 1,6-bisphosphatase (FBPase) (EC 3.1.3.11): 50 mM imidazole, pH 7.1, at 20°C, 15 mM $MgCl_2$, 0.1 mM FBPase, 0.2 mM NADP, 2 units G6PDH, 10 units phosphoglucose isomerase (PGI).

Glucose-6-phosphate dehydrogenase (G6PDH) (EC 1.1.1.49): 50 mM imidazole, pH 7.1, at 20°C, 7 mM $MgCl_2$, 0.4 mM NADP, 1 mM glucose-6-phosphate.

Glutamate pyruvate transaminase (GPT) (EC 2.6.1.2): 50 mM imidazole, pH 7.1, at 20°C, 200 mM L-alanine, 0.2 mM NADH, 0.025 mM pyridoxal phosphate, 1 unit LDH, 10.5 mM α-ketoglutarate.

Glutamate oxaloacetate transaminase (GOT) (EC 2.6.1.1): 50 mM imidazole, pH 7.1, at 20°C, 0.2 mM NADH, 0.25 mM pyridoxal phosphate, 30 mM aspartate, 7 mM α-ketoglutarate, 1 unit malate dehydrogenase (MDH).

Glutamate dehydrogenase (GDH) (EC 1.4.1.2): 50 mM imidazole, pH 8.1, at 20°C, 250 mM ammonium acetate, 0.1 mM EDTA, 0.1 mM NADH, 1 mM ADP, 14 mM α-ketoglutarate.

Citrate synthase (CS) (EC 4.1.3.7): 50 mM imidazole, pH 8.0, at 20°C, 0.1 mM 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), 0.3 mM acetyl-CoA, 0.5 mM oxaloacetate.

Carnitine octanoyl transferase (COT) (EC 2.3.1.21): 50 mM imidazole, pH 8.1, at 20°C, 5 mM L-carnitine, 1 mM DTNB, 50 μM octanoyl-CoA.

Carnitine palmitoyl transferase (CPT) (EC 2.3.1.21): 50 mM imidazole, pH 8.1, at 20°C, 5 mM L-carnitine, 1 mM DTNB, 50 μM palmitoyl-CoA.

β-Hydroxybutyrate dehydrogenase (BHBDH) (EC 1.1.1.30): 50 mM imidazole, pH 8.1, at 20°C, 2 mM NAD, 10 mM β-hydroxybutyrate, 0.3 μg rotenone/L.

3-Hydroxyacyl-CoA dehydrogenase (HOAD) (EC 1.1.1.35): 50 mM imidazole, pH 8.1, at 20°C, 0.2 mM acetoacetyl-CoA, 0.1 mM NADH.

Acetoacetyl-CoA thiolase (AA-CoA thiolase) (EC 2.3.1.9): 50 mM imidazole, pH 7.2, at 20°C, 5 mM $MgCl_2$, 0.12 mM CoA, 0.07 mM acetoacetyl-CoA, measured at 303 nM.

3-Oxoacid transferase (OAT) (EC 2.8.3.5): 50 mM imidazole, pH 7.2, at 20°C, 5 mM iodoacetamide, 0.4 mM succinyl-CoA, 5 mM $MgCl_2$, 50 mM acetoacetate, measured at 313 nM.

Hydroxymethylglutaryl-CoA lyase (HMG-CoA lyase) (EC 4.1.3.4): 50 mM imidazole, pH 8.0, at 20°C, 10 mM $MgCl_2$, 0.2 mM NADH, 0.2 mM HMG-CoA, 1 unit BHBDH.

Statistics

Within each tissue and for each enzyme, an analysis of variance of activities was performed among control, full-strength seawater, and low-salinity treated groups using the general linear models procedure of the SAS (Statistical Analysis System) Institute Inc., Box 8000, Cary, NC 27511-8000. Mean enzyme activities were compared using Scheffe's multiple comparison technique with the probability of making a type I error set at 0.05.

Chemicals

All chemicals and biochemicals were obtained from the Sigma Chemical Co., St. Louis, MO, and were of the highest purity available.

[1]EC refers to the system of nomenclature recommended by the Commission on Biochemical Nomenclature, 1972.

Results

Gill

There were significant differences between the initial activities and the activities after 4–6 wk at the same salinity (1000 mosmol/L) of HK ($p < 0.0001$)⟨ FBPase ($p < 0.0018$), GDH ($p < 0.0158$), and HOAD ($p < 0.004$) (Table 1). There were significantly lower activities of HK ($p < 0.0001$), FBPase ($p < 0.0018$), and HOAD ($p < 0.004$) in the low-salinity exposed group compared with the group maintained in full-strength seawater salinity (Table 1). Compared with other tissues, OAT and G6PDH activities were highest in all treatment groups. CS was highest in gill compared with other tissues in control and full-strength seawater and low-salinity treated animals but not in control animals. The gill had the highest GDH activity compared with other tissues only in low-salinity animals.

Hepatopancreas

There were significant differences between the initial activities and the activities after 4–6 wk at the same salinity (1000 mosmol/L) of HK ($p < 0.0001$), FBPase ($p < 0.0001$), GDH ($p < 0.0005$), and HOAD ($p < 0.0001$) (Table 2). Significantly lower activities of HK ($p < 0.0001$), FBPase, and HOAD ($p < 0.0001$) were measured in the low-salinity exposed group compared with the group maintained in full-strength seawater (Table 2). The highest activities of HOAD, COT, and CPT were found in this tissue in all groups. HK and AA-CoA thiolase were highest in hepatopancreas compared with other tissues in control (full-strength seawater) and low-salinity animals. Hepatopancreas had the highest GOT and GPT but not GDH compared with other tissues in full-strength seawater animals. The highest FBPase levels in control and full-strength seawater but not low-salinity animals were observed in hepatopancreas compared with other tissues.

Mantle

There were significant differences between the initial activities and the activities after 4–6 wk at the same salinity (1000 mosmol/L for HK ($p < 0.0001$), FBPase ($p < 0.0001$), CS ($p < 0.0007$), and HOAD ($p < 0.0033$) (Table 3). As well, there were significantly lower activities for HK ($p < 0.0001$), FBPase ($p < 0.0001$), and CS ($p < 0.0007$) in the low-salinity exposed group compared with the group maintained in full-strength seawater (Table 3). BHBDH could not be detected in any of the tissues (activity < 0.003 U/g).

Adductor Muscle

There were significant differences between the initial activities and the activities after 4–6 wk at the same salinity (1000 mosmol/L) of PK ($p < 0.0033$) and HOAD ($p < 0.0083$) (Table 4). There were significantly lower ($p < 0.0083$) activities of HOAD in the low-salinity exposed group compared with the group maintained in full-strength seawater (Table 4). The highest HMG-CoA lyase activities were found in this tissue in full-strength seawater and low-salinity treated animals compared with other tissues. GPT, GOT, and GDH were highest in adductor muscle compared with other tissues in control and low-salinity treated animals but not in full-strength seawater animals. Adductor muscle had the highest FBPase activity in low-salinity animals compared with other tissues.

Discussion

Metabolic Organization of the Oyster

All tissues examined demonstrated HOAD, COT, and CPT activity, indicative of the ability to oxidize fatty acids. Lipid oxidation may play an important role in the metabolism of most tissues of the oyster. This contrasts with the apparent inability of the molluscan heart to oxidize lipids (Ballantyne et al. 1981; Ballantyne and Storey 1983). The levels of COT and CPT indicate fatty acid oxidation proceeds in a carnitine-dependent manner in agreement with our previous studies of acyl carnitine oxidation in mitochondria isolated from hepatopancreas of other species of bivalves (*Mercenaria mercenaria*, Ballantyne and Storey 1984; *Mytilus edulis*, Ballantyne and Moon 1985) and gill of *Crassostrea virginica* (Ballantyne and Moyes 1987a). The hepatopancreas has the highest levels of these enzymes,

TABLE 1. Activities of enzymes of intermediary metabolism in the gill of the oyster (*Crassostrea virginica*). Values are means ± SD. The number of determinations is given in parentheses after each value. Units are micromoles of substrate converted to product per minute per gram wet weight of tissue. Similar letters (a, b, or c) after the parentheses indicate those values not significantly different as described in Materials and Methods.

Enzyme	Initial	Low-salinity acclimation	Full-strength seawater
HK	0.077 ± 0.104 (6) a	0.142 ± 0.142 (6) a	0.431 ± 0.118 (6) b
PK	1.138 ± 1.674 (6) a	0.368 ± 0.124 (6) a	0.366 ± 0.174 (6) a
FBPase	0.019 ± 0.104 (7) a	0.040 ± 0.020 (8) a	0.078 ± 0.023 (6) b
G6PDH	2.226 ± 0.553 (6) a	1.836 ± 0.344 (6) a	2.376 ± 0.339 (6) a
GPT	1.147 ± 0.430 (6) a	1.377 ± 0.279 (6) b	1.932 ± 0.506 (6) ab
GOT	6.148 ± 1.872 (6) a	4.272 ± 1.003 (6) a	4.763 ± 1.627 (6) a
GDH	0.342 ± 0.045 (6) a	0.213 ± 0.054 (6) b	0.200 ± 0.053 (6) b
CS	2.378 ± 0.868 (6) a	1.558 ± 0.765 (6) a	2.676 ± 0.743 (6) a
COT	0.020 ± 0.017 (5) a	0.015 ± 0.006 (3) a	0.037 ± 0.007 (5) a
CPT	0.015 ± 0.034 (4) a	0.034 ± 0.014 (7) a	0.041 ± 0.014 (4) a
HOAD	0.159 ± 0.033 (6) a	0.190 ± 0.043 (6) a	0.301 ± 0.087 (6) b
AA-CoA thiolase	0.081 ± 0.047 (6) a	0.116 ± 0.049 (8) a	0.060 ± 0.032 (6) a
OAT	0.028 ± 0.010 (6) a	0.015 ± 0.012 (8) a	0.015 ± 0.007 (6) a
HMG-CoA lyase	0.011 ± 0.013 (6) a	0.030 ± 0.013 (8) b	0.025 ± 0.020 (5) ab
BHBDH	<0.003	<0.003	<0.003

TABLE 2. Activities of enzymes of intermediary metabolism in the hepatopancreas of the oyster (*Crassostrea virginica*). Values are means ± SD. The number of determinations is given in parentheses after each value. Units are micromoles of substrate converted to product per minute per gram wet weight of tissue. Similar letters (a, b, or c) after the parentheses indicate those values not significantly different as described in Materials and Methods.

Enzyme	Initial	Low-salinity acclimation	Full-strength seawater
HK	0.262 ± 0.059 (8) a	0.217 ± 0.068 (6) a	0.442 ± 0.064 (6) b
PK	1.602 ± 1.391 (6) a	0.395 ± 0.133 (6) a	0.522 ± 0.242 (6) a
FBPase	0.044 ± 0.022 (6) a	0.051 ± 0.034 (8) a	0.164 ± 0.022 (5) b
G6PDH	1.550 ± 0.332 (6) a	0.984 ± 0.164 (6) a	1.565 ± 0.689 (6) a
GPT	2.562 ± 1.473 (6) a	2.171 ± 1.552 (6) a	4.763 ± 1.769 (5) a
GOT	6.408 ± 2.021 (6) a	4.272 ± 1.003 (6) a	5.412 ± 0.834 (6) a
GDH	0.168 ± 0.037 (6) a	0.088 ± 0.019 (6) b	0.083 ± 0.029 (6) b
CS	1.995 ± 0.643 (6) a	1.775 ± 0.430 (5) a	2.127 ± 0.579 (6) a
COT	0.024 ± 0.034 (6) a	0.031 ± 0.017 (5) a	0.054 ± 0.025 (6) a
CPT	0.044 ± 0.034 (3) a	0.081 ± 0.033 (4) a	0.056 ± 0.033 (6) a
HOAD	0.194 ± 0.062 (6) a	0.241 ± 0.104 (6) a	0.597 ± 0.117 (6) b
AA-CoA thiolase	0.165 ± 0.056 (8) a	0.198 ± 0.121 (8) a	0.236 ± 0.144 (6) a
OAT	0.019 ± 0.007 (6) a	0.011 ± 0.014 (4) a	0.013 ± 0.009 (5) a
HMG-CoA lyase	0.044 ± 0.018 (6) a	0.028 ± 0.031 (4) a	0.039 ± 0.017 (6) a
BHBDH	<0.003	<0.003	<0.003

TABLE 3. Activities of enzymes of intermediary metabolism in the mantle of the oyster (*Crassostrea virginica*). Values are means ± SD. The number of determinations is given in parentheses after each value. Units are micromoles of substrate converted to product per minute per gram wet weight of tissue. Similar letters (a, b, c) after the parentheses indicate those values not significantly different as described in Materials and Methods.

Enzyme	Initial	Low-salinity acclimation	Full-strength seawater
HK	0.134 ± 0.090 (5) a	0.032 ± 0.018 (5) c	0.339 ± 0.048 (6) b
PK	1.748 ± 2.769 (6) a	0.304 ± 0.097 (6) a	0.276 ± 0.131 (6) a
FBPase	0.027 ± 0.022 (5) a	0.030 ± 0.009 (8) a	0.093 ± 0.012 (6) b
G6PDG	1.304 ± 0.210 (6) a	1.073 ± 0.307 (5) a	1.556 ± 0.389 (6) a
GPT	0.943 ± 0.433 (4) a	1.226 ± 0.248 (6) a	1.685 ± 0.580 (6) a
GOT	5.947 ± 1.035 (4) a	4.341 ± 1.040 (6) a	5.078 ± 0.621 (6) a
GDH	0.273 ± 0.135 (4) a	0.124 ± 0.049 (6) a	0.269 ± 0.319 (6) a
CS	1.594 ± 0.304 (6) a	0.808 ± 0.341 (6) a	1.652 ± 0.250 (6) b
COT	0.006 ± 0.000 (1) a	0.005 ± 0.003 (3) a	0.020 ± 0.011 (6) a
CPT	0.008 ± 0.003 (5) a	0.010 ± 0.006 (4) a	0.026 ± 0.012 (5) a
HOAD	0.086 ± 0.018 (6) a	0.131 ± 0.044 (6) ab	0.176 ± 0.037 (6) b
AA-CoA thiolase	0.041 ± 0.021 (6) a	0.040 ± 0.030 (7) a	0.057 ± 0.026 (6) a
OAT	0.014 ± 0.006 (6) a	0.012 ± 0.005 (7) a	0.013 ± 0.007 (6) a
HMG-CoA lyase	0.031 ± 0.012 (6) a	0.019 ± 0.012 (7) a	0.028 ± 0.021 (6) a
BHBDG	<0.003	<0.003	<0.003

suggesting a substantial reliance on lipid as a metabolic substrate. The hepatopancreas of the oyster contains large stores of lipids, especially triglycerides (Swift et al. 1980), which could be used to supply endogenous energy requirements in addition to serving as a central storage tissue.

The levels of HK indicate that all the tissues examined are capable of using exogenous glucose to some extent. Values of HK reported for oyster adductor are similar to those determined in adductor of *Mytilus edulis* (Crabtree and Newsholme 1972) and *Ostrea edulis* (Zammit and Newsholme 1976).

CS levels indicate that gill has a slightly higher aerobic capacity compared with other tissues.

Ketone bodies have several functions in intermediary metabolism which have not been investigated in marine bivalves: (1) during starvation, higher vertebrate tissues, which normally rely extensively on glucose, use ketone bodies; (2) ketone bodies are involved in cholesterol synthesis; and (3) ketone bodies may be important in the transport of lipid carbon between tissues in marine bivalves (Ballantyne et al. 1987). The absence of detectable BHBDH activity agrees with the findings of Beis et al. (1980) in a variety of invertebrates and the very low mitochondrial oxidation of β-hydroxybutyrate in oyster gill mitochondria (Ballantyne and Moyes 1987a). Acetoacetate seems to be the predominant ketone body in marine invertebrates. The presence of OAT (the first enzyme in the pathway of acetoacetate utilization) indicates that acetoacetate can be used by all the tissues examined. The activities of OAT in oyster adductor are about 100-fold lower than those found in a more active marine mollusc, *Buccinum* (Beis et al. 1980). Levels of AA-CoA thiolase were also lower than those reported for the radular retractor muscle of the common whelk (*Buccinum undatum*) by Beis et al. (1980). This is likely due to the lower assay temperature in the present study and the lower metabolic rate of sessile bivalves. Based on tissue

TABLE 4. Activities of enzymes of intermediary metabolism in the adductor of the oyster (*Crassostrea virginica*). Values given means ± SD. The number of determinations is given in parentheses after each value. Units are micromoles of substrate converted to product per minute per gram wet weight of tissue. Similar letters (a, b, or c) after the parentheses indicate those values not significantly different as described in Materials and Methods.

Enzyme	Initial	Low-salinity acclimation	Full-strength seawater
HK	0.167 ± 0.035 (8) a	0.161 ± 0.093 (6) a	0.224 ± 0.049 (6) a
PK	1.138 ± 0.568 (6) a	0.350 ± 0.095 (6) b	0.356 ± 0.169 (6) b
FBPase	0.038 ± 0.038 (7) a	0.109 ± 0.061 (7) b	0.072 ± 0.032 (6) ab
G6PDH	0.855 ± 0.105 (6) a	0.634 ± 0.072 (6) a	0.817 ± 0.213 (6) a
GPT	3.021 ± 0.731 (5) a	3.127 ± 1.144 (6) a	3.167 ± 1.191 (6) a
GOT	7.363 ± 1.872 (5) a	5.693 ± 1.003 (6) a	5.343 ± 1.627 (6) a
GDH	0.386 ± 0.226 (5) a	0.166 ± 0.097 (6) b	0.180 ± 0.015 (6) ab
CS	1.604 ± 0.557 (6) a	1.194 ± 0.195 (6) a	1.383 ± 0.326 (6) a
COT	0.013 ± 0.008 (5) a	0.010 ± 0.005 (3) a	0.013 ± 0.006 (4) a
CPT	0.013 ± 0.014 (6) a	0.039 ± 0.023 (3) a	0.022 ± 0.013 (4) a
HOAD	0.115 ± 0.013 (6) a	0.130 ± 0.041 (6) a	0.195 ± 0.046 (6) b
AA-CoA thiolase	0.034 ± 0.015 (6) a	0.040 ± 0.018 (8) a	0.040 ± 0.013 (6) a
OAT	0.010 ± 0.004 (6) a	0.012 ± 0.009 (7) a	0.008 ± 0.002 (5) a
HMG-CoA lyase	0.025 ± 0.009 (6) a	0.035 ± 0.016 (8) a	0.045 ± 0.023 (6) a
BHBDH	<0.003	<0.003	<0.003

HMG-CoA lyase activities, all tissues examined have the capability to synthesize cholesterol from ketone bodies.

Amino acids are important intracellular compatible osmolytes in oyster tissues (Lynch and Wood 1966) but also serve as important oxidative substrates (Ballantyne and Storey 1983, 1985; Ballantyne and Moyes 1987b, 1987c; Ballantyne et al. 1987). GDH activities are low but similar to those reported for *Mytilus edulis* tissues (Addink and Veenhof 1975). In spite of the low activities of GDH in tissues of bivalve molluscs, this enzyme is likely important in the metabolism of amino acids during salinity stress (Moyes et al. 1985). GPT activity in mantle is similar to that reported by Awapara and Campbell (1964) for the oyster mantle. GOT activities were highest of all the enzymes examined in all tissues and in adductor are similar to those reported by Zammit and Newsholme (1976) for *Ostrea edulis* adductor muscle. The high GOT activities likely reflect the mechanism for the anaerobic production of succinate from aspartate, as has been demonstrated in oyster heart (Collicutt and Hochachka 1977).

Time-Dependent Changes in Enzyme Levels

A comparison of the changes in the full-strength seawater group from the onset of the experiment to the end of the treatment period indicates increases in HOAD activity in all tissues and FBPase and HK in all tissues except adductor. Taken together, this suggests increased fatty acid oxidation and gluconeogenesis in most tissues. Increased fatty acid oxidation may have a sparing effect on carbohydrate. Increased HK levels suggest that elevated glucose uptake in most tissues may indicate normal seasonal increases in glycogen synthesis. Wild populations of marine mussels accumulate glycogen over the same time period as the present study (Thompson 1984). Some metabolic readjustment to a new diet and laboratory conditions has, therefore, occurred. The decrease in GDH in gill and hepatopancreas suggests reduced catabolism of amino acids in these tissues, perhaps due to altered diet.

Effects of Salinity Exposure

Based on the present study, no major metabolic reorganization is apparent after low-salinity exposure for 4–6 wk. This occurs in spite of the substantial changes in intracellular organic solute content known to occur as euryhaline molluscs adapt to changing salinities (see Moyes and Ballantyne 1987 for review). It is likely that transitory changes in enzyme levels are responsible for the changing intracellular solute environment and that these are essentially complete after 4 wk.

Regulation of amino acid concentrations with salinity acclimation must require transitory changes in enzyme activity to achieve new steady-state levels. The lack of effect of exposure to different salinities on GPT activity agrees with the findings of Dupaul and Webb (1974), who found that the levels of this enzyme increased in the initial hours of high-salinity acclimation and then returned to normal levels in the subsequent hours and days. While Wickes and Morgan (1976) found that GOT and GDH varied linearly with salinity in populations of oysters from different salinities, such differences may reflect genetic heterogeneity rather than acclimation responses. The highest GDH in low-salinity animals occurred in gill. Greater GDH function in this tissue in low-salinity animals identifies this tissue as the major site of deamination in low salinity. Higher rates of ammonia/ammonium excretion are correlated with losses of amino acids from tissues of bivalves in low salinity (Bartenberger and Pierce 1976). GDH is known to be activated by increasing ionic strength in crustaceans (Gilles 1974; Schoffeniels 1976), but we have found oyster GDH to be inhibited by increasing ionic strength (Ballantyne and Moyes 1987c).

The lack of effect of exposure to high and low salinities on maximal activity of PK agrees with the study of Wickes and Morgan (1976) in naturally acclimated oyster adductor muscle. Other kinetic properties of PK have been shown to change with salinity acclimation in *Mytilus edulis* (Siebenaller 1979). Such changes may be involved in maintenance of metabolic homeostasis under altered intracellular conditions.

The reduced FBPase in gill, hepatopancreas, and mantle of low-salinity treated animals may indicate reduced gluconeogenesis in these tissues due to a lower glucose or glycogen requirement at these salinities. Correspondingly lower HK in each of these tissues suggests reduced tissue uptake of glucose. Taken together, these findings suggest diminished carbohydrate requirements at low salinity. It is likely that carbohydrate

is conserved during periods of adequate oxygen availability (Hochachka and Somero 1984). Ballantyne and Moon (1985) reported very low oxidation of pyruvate by mitochondria isolated from the hepatopancreas of *Mytilus edulis*, indicating that carbohydrate oxidation may normally be suppressed.

The reduced activity of HOAD in low-salinity exposed adductor muscle (30% decrease), gill (40% decrease), and hepatopancreas (50% decrease) may indicate reduced reliance on lipid as an energy source at low salinity. A rationale for this observation remains to be determined.

Exposure to high and low salinities did not affect the activity of any of the enzymes associated with ketone body metabolism. Acetoacetate oxidation by isolated oyster gill mitochondria was not affected by salinity acclimation or osmolarity (Ballantyne and Moyes 1987a). An increase in the activity of HMG-CoA lyase was observed in gill with time, but activities were not affected by exposure to high or low salinities. Bivalve molluscs can synthesize cholesterol from acetate (see Voogt 1983 for review). Cholesterol is an important component of most membranes, and it changes in distribution and abundance under various environmental conditions to maintain membrane fluidity. The sterol content of *Crassostrea* changes with reproductive state (Swift et al. 1980). While the cholesterol content of membranes changes with salinity acclimation in the gills of the guppy *Poecilia reticulata* (Daikoku et al. 1982), it is not known if salinity affects the cholesterol content of marine mollusc membranes.

While the changes in metabolism outlined above may explain some of the differences between the full-strength seawater and the low-salinity groups, another explanation must be considered. Studies of oyster gill mitochondria indicate that elevated rates of oxidation at low salinities are due to reduced matrix ionic strength (Ballantyne and Moyes 1987c). It has been argued (Ballantyne and Chamberlin 1988) that enhancement of enzyme activity could occur through dilution of the intracellular inorganic ion levels. Salinity acclimation does alter the osmotic properties of isolated oyster gill mitochondria (Ballantyne and Moyes 1987b) in a manner which would tend to counteract the increased oxidation rates at low salinities. It would appear from the present study that maintenance of normal metabolic function at low salinities may involve reduced enzyme activities. This occurred in the present study for HK and CS in mantle, HK, FBPase, and HOAD in gill and adductor, and HOAD in adductor. CS activity has been shown to be inhibited by increasing ionic strength in pig heart (Wu and Yang 1970), a crustacean and sea anemone (Sarkissian and Boatwright 1974), as well as the oyster (Sarkissian 1974). The lower intracellular ionic strength in low-salinity treated oyster tissues would increase intracellular activity, necessitating a reduction in enzyme levels to compensate for this. HOAD has been found to be influenced by ionic strength in crustaceans (Chapelle and Dandrifosse 1972). In these organisms, only unphysiologically high ionic strength was inhibitory. Studies of the effects of ionic strength on molluscan HOAD are needed.

Not all enzymes have responded in the same fashion to reduced salinity due to the variation in the responses of specific enzymes to changing ionic strength. The role of ionic strength and specific ion changes in mediating metabolic changes in response to changing salinity in osmoconforming organisms requires further investigation. Based on the present study, it appears that before the significance of metabolic changes in enzyme activities can be assessed, the sensitivity of each to changing intracellular ionic strength and specific ion levels must be determined.

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