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THE EFFECTS OF SALINITY ACCLIMATION ON THE OSMOTIC PROPERTIES OF MITOCHONDRIA FROM THE GILL OF *CRASSOSTREA VIRGINICA*

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SUMMARY

Glutamate oxidation in mitochondria from the gills of oysters (*Crassostrea virginica* Gmelin) acclimated to sea water (SW) is sensitive to assay medium osmolarity. Compared to a medium of the osmolarity of sea water, decreasing osmolarity stimulates glutamate oxidation and increasing osmolarity inhibits glutamate oxidation. Glutamate oxidation by mitochondria from oysters acclimated to dilute sea water (DSW) is 2·5-fold greater than in SW animals when each is assayed under isosmotic conditions. The maximal rates obtained in both acclimation groups are equal. Although the DSW animals were acclimated to an osmolarity approximately 660 mosmol l^{-1} lower than SW animals, the osmotic optimum was only 100–200 mosmol l^{-1} lower in the former group. Isolation medium osmolarity does not affect the osmolarity at which the maximum rate of glutamate oxidation is observed in either acclimation group. A low-osmolarity isolation medium reduces the oxidation rates and quality of the mitochondria, as assessed by the respiratory control ratio in both acclimation groups. These data suggest that an optimal matrix solute concentration or ionic strength is required for maximal rates of glutamate oxidation rather than an optimal mitochondrial volume. The optimal mitochondrial matrix milieu can be achieved both by acclimation to dilute sea water and by incubation in hypo-osmotic media.

INTRODUCTION

Amino acids are important intracellular osmolytes in marine osmoconforming bivalve molluscs. Tissue amino acid levels decrease in animals exposed to hypo-osmotic sea water, as part of a volume regulatory mechanism. This occurs without observable increases in excretion of intact amino acids into the environment, suggesting that amino acids are catabolized at greater rates under these conditions (Livingstone, Widdows & Fieth, 1979; Henry & Mangum, 1980). Support for this

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mechanism is provided by the observation of elevated haemolymph ammonia levels and higher rates of ammonia excretion (Strange & Crowe, 1979).

All of the quantitatively important amino acids (glutamate, alanine, glycine) are catabolized by mitochondrial pathways. Osmolarity has been shown to affect the rates of oxidation of amino acids by mitochondria from a variety of osmoconformers, including several species of bivalves (Ballantyne & Storey, 1983, 1985; Ballantyne & Moon, 1985) and a marine elasmobranch (Ballantyne & Moon, 1986; Ballantyne, Moyes & Moon, 1986; Moyes, Moon & Ballantyne, 1986). As mitochondria act as perfect osmoconformers (Sorgo, Zhang & Tedeschi, 1985), changes in intracellular osmolarity cause similar changes in intramitochondrial osmolarity and probably alter matrical inorganic ion levels. It has been suggested that osmotically induced changes in mitochondrial oxidation rates are due to altered concentrations of matrical ions (Joseph, McGivan & Meijer, 1981; Ballantyne & Moyes, 1987). Changing intracellular inorganic ion levels during salinity acclimation may affect the mitochondrial matrix ion content and, thereby, the osmotic sensitivity of metabolite oxidation.

The present experiments were carried out to determine the effects of acclimation salinity on the osmotically sensitive mitochondrial oxidation of glutamate. To ensure that effects of salinity acclimation were not an artefact associated with the method of isolation the effects of isolation medium osmolarity were also examined.

MATERIALS AND METHODS

Animals

Malpeque oysters (*Crassostrea virginica*) were obtained from a local seafood supplier and maintained in recirculated artificial sea water (1000 mosmol l^{-1}) at 10°C in the Marine Biology Laboratory at the University of Guelph for 1 week before salinity acclimation. The animals were divided into two groups. One group was kept in normal sea water for 4 weeks (SW) and the other was placed directly into dilute (333 mosmol l^{-1}) sea water (DSW) at 10°C for the same period. Acclimation to low salinity requires up to 4 weeks in this species (Heavers & Hammen, 1985). Both groups opened their valves within 1 day of exposure to the acclimation salinity. The mortality over the 4-week acclimation period was less than 2%.

Isolation of mitochondria

The isolation medium consisted of 300 mmol l^{-1} sucrose, 50 mmol l^{-1} KCl, 50 mmol l^{-1} NaCl, 30 mmol l^{-1} Hepes (pH 7·20 at 20°C), 8 mmol l^{-1} EGTA, 1% bovine serum albumin (BSA, essentially fatty acid free). The osmolarity of the isolation medium was reduced by omitting sucrose (low-osmolarity isolation medium).

Gills of six or seven animals were removed, blotted dry and placed in 10 ml of ice-cold isolation medium. The tissue was homogenized with three passes of a Potter–Elvehjem homogenizer equipped with a loosely fitting Teflon pestle. The homogenate was centrifuged for 10 min at 200 ***g***. The supernatant was then centrifuged at 6560 ***g*** for 10 min. The resulting mitochondrial pellet was resuspended

in the ice-cold isolation medium to give a mitochondrial protein content of 10–20 mg ml^{-1}.

Mitochondrial oxidation

Oxygen uptake by mitochondria was measured in 1-ml glass cells using Clarke-type oxygen electrodes. Temperature was maintained constant at $10 \pm 0{\cdot}1$ °C with a Haake refrigerated water circulator. Nine volumes of assay medium were mixed with one volume of isolation medium containing the mitochondria. The assay medium consisted of 30 mmol l^{-1} Hepes, 10 mmol l^{-1} KH_2PO_4 (pH 7·20 at 20°C), 1 % BSA and varying amounts of sucrose. Assays were completed within 2 h of isolation of the mitochondria. Respiratory states 3 and 4 were determined as described by Chance & Williams (1956) using saturating amounts (10–20 mmol l^{-1}) of glutamate as the substrate and the respiratory control ratios were determined as described by Estabrook (1967) using 100 μmol l^{-1} ADP. Permeability of the mitochondria was monitored using the oxidation of exogenous NADH (100 μmol l^{-1}).

Protein concentration

Protein concentration was measured using a modified Biuret procedure with 10 % deoxycholic acid added to solubilize the mitochondria (Gornall, Bardawill & Davis, 1949). BSA was used as the standard. Protein content of the isolation medium for each batch was subtracted from the total protein content of the mitochondrial suspension to determine the mitochondrial protein concentration.

Chemicals and statistical analysis

All chemicals were obtained from the Sigma Chemical Co., St Louis, MO. Where statistical comparisons were made, a two-tailed Student's t-test was used.

RESULTS

When assayed in reaction media isosmotic for each acclimation group, the maximum rate of state-3 glutamate oxidation by the DSW group was 2·5-fold greater than by the SW group. The highest state-3 rates of oxidation of glutamate for both acclimation groups were obtained when the mitochondria were isolated in the high-osmolarity medium (Fig. 1A). When both acclimation groups were isolated in media of equal osmolarities the state-3 rate of oxidation of the SW group was displaced 100–200 mosmol l^{-1} higher than the profile for the DSW group (Fig. 1A). This trend was observed when mitochondria were isolated in either the high- or the low-osmolarity medium. There was no significant difference between the maximal state-3 rates for the two acclimation groups isolated in the high-osmolarity medium, but these peaks occurred about 100 mosmol l^{-1} apart.

The state-3 rates (Fig. 1A) of mitochondria isolated in lower osmolarity medium (238 mosmol l^{-1}) were consistently lower ($P < 0{\cdot}05$) at each osmolarity than those values obtained with mitochondria isolated in high-osmolarity medium (538 mosmol l^{-1}). Isolation medium osmolarity had no effect on the osmotic peak in either

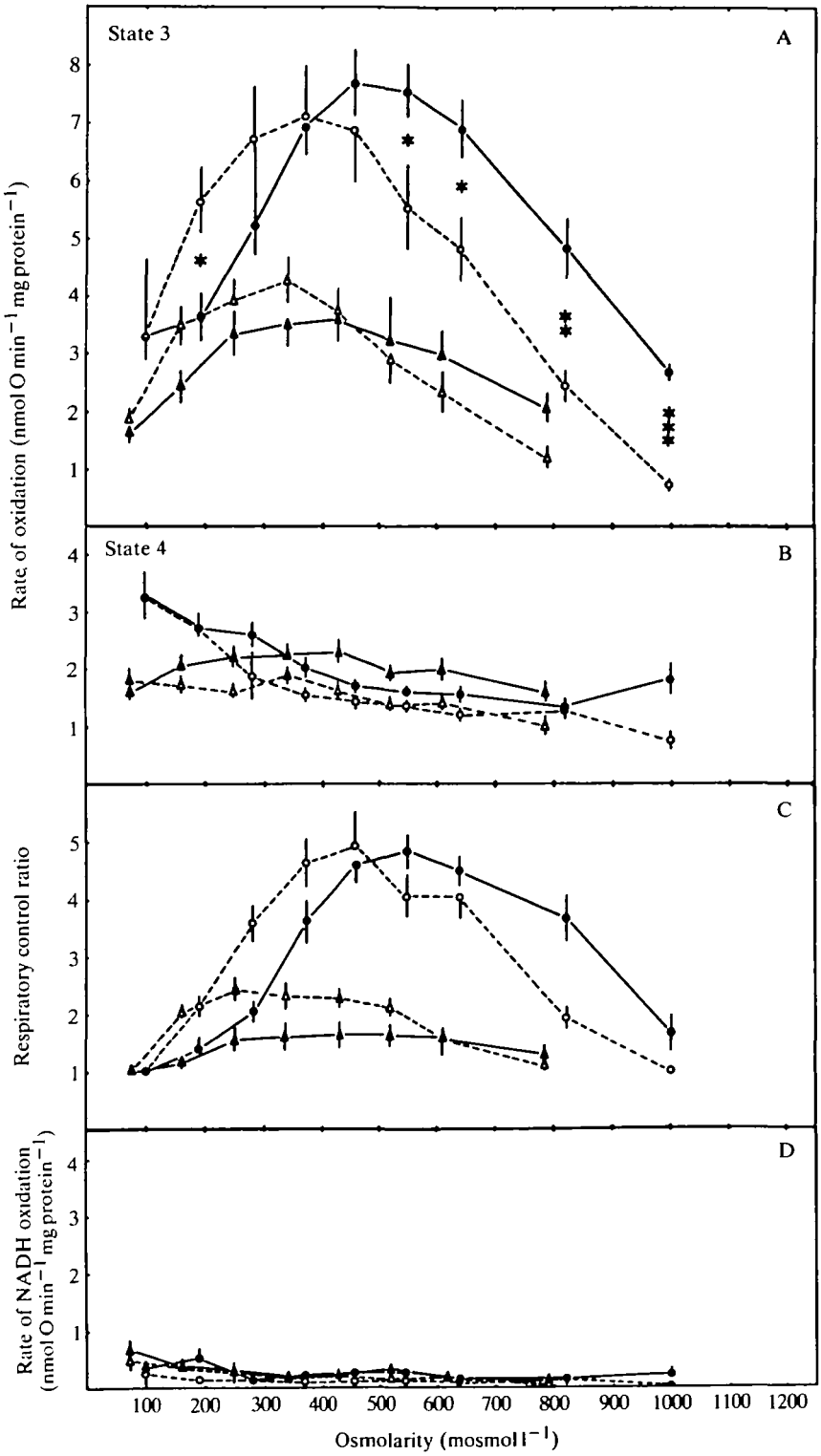
State 3
A
State 4
B
C
D
Rate of oxidation (nmol O min^{-1} mg protein^{-1})
Respiratory control ratio
Rate of NADH oxidation (nmol O min^{-1} mg protein^{-1})
Osmolarity (mosmol l^{-1})
100 200 300 400 500 600 700 800 900 1000 1100 1200

Fig. 1

acclimation group. The osmotic profiles were much broader for both groups when isolated in the low-osmolarity isolation medium.

Mitochondria from SW oysters had higher state-4 rates of oxidation ($P < 0{\cdot}005$) compared with mitochondria isolated from DSW animals when both groups were isolated in high-osmolarity medium (Fig. 1B). Mitochondria from SW animals had higher state-4 rates when isolated in low-osmolarity medium instead of in high-osmolarity medium.

The osmotic profiles for the respiratory control ratio (RCR) values for all groups were broader than those for the state-3 rates (Fig. 1C). The RCR values obtained for the mitochondria isolated from both groups were parallel to the state-3 rates of oxidation. The RCR values were lower ($P < 0{\cdot}05$) for both acclimation groups when isolated in the low-osmolarity medium.

The permeability of the mitochondria, as assessed by the rates of oxidation of exogenous NADH (Fig. 1D), was not affected by the acclimation conditions, the osmolarity of the isolation medium or the osmolarity of the assay medium.

DISCUSSION

Acclimation to dilute sea water results in lower intracellular inorganic ion levels in the muscle of the oyster, *Ostrea edulis* (Bricteux-Grégoire, Duchâteau-Bosson, Jeuniaux & Florkin, 1964). Tissue ion levels in oysters acclimated to different salinities probably reflect mitochondrial ion levels, as in other species (Chen, Greenawalt & Lehninger, 1974; Sorgo *et al.* 1985). In seawater-acclimated oysters, the activity of the glutamate oxidative pathway in gill mitochondria is dependent on ionic strength in the mitochondrial matrix (Ballantyne & Moyes, 1987). An enhanced rate of glutamate oxidation can also be achieved with acclimation to dilute sea water.

In the present study, mitochondria from oysters acclimated to DSW showed greater rates of glutamate oxidation than mitochondria isolated from oysters acclimated to SW when each group was assayed at an isosmotic osmolarity. As the maximal rates of glutamate oxidation were not affected by acclimation osmolarity, changes in activities of the glutamate oxidative enzymes were probably not responsible for the greater rates of glutamate oxidation in SW animals. It has been

Fig. 1. Osmotic effects on oxidation by mitochondria isolated from the gills of *Crassostrea virginica* exposed to full-strength (1000 mosmol l^{-1}) and one-third-strength (333 mosmol l^{-1}) sea water. Osmolarity was varied using sucrose. Values are means ± S.E. for six determinations. The asterisks between error bars indicate the level of significance of the difference between the means. $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}005$; $^{***}P < 0{\cdot}0005$. Closed symbols and solid lines denote means obtained from animals acclimated to full-strength sea water. Open symbols and dashed lines denote means obtained from mitochondria isolated from animals acclimated to the low salinity. Triangular symbols indicate means obtained from mitochondria isolated in the low-osmolarity medium and circular symbols indicate means obtained from mitochondria isolated in the higher osmolarity medium. Where no error bars appear, the standard error is smaller than the size of the symbol. (A) The effects of osmolarity on the state-3 oxidation of glutamate. (B) The effects of osmolarity on the state-4 oxidation of glutamate. (C) The effects of osmolarity on the respiratory control ratio for the oxidation of glutamate. (D) The effects of osmolarity on the oxidation of NADH.

suggested that osmotically induced swelling of isolated mitochondria changes lipid membrane structure (Atsmon & Davis, 1967; Holtzman *et al.* 1978). If hypo-osmotically induced stimulation of glutamate oxidation is dependent on physical changes in membrane structure associated with swelling, then one would expect increased rates of glutamate oxidation to be sustained *in vivo* only as long as the mitochondria are swollen. Maximal rates of glutamate oxidation can only be achieved in SW-acclimated animals with hypo-osmotic swelling. Swelling dilutes the matrical environment, bringing conditions towards the optimum for glutamate oxidation. In animals acclimated to a lower salinity, acclimation alone appears to be sufficient to achieve a mitochondrial matrix composition near the optimum for glutamate oxidation. Incubation in either hyperosmotic media (increasing solute concentration and ionic strength) or hypo-osmotic media (decreasing solute concentration or ionic strength) reduces the rate of oxidation. The effects of osmolarity on oysters acclimated to different salinities are, therefore, consistent with the concept of an optimal matrix ionic strength (Ballantyne & Moyes, 1987) rather than with changing physical properties of the inner mitochondrial membrane.

Mitochondria isolated in the low-osmolarity isolation medium had lower RCR values due to lower state-3 rates, regardless of the acclimation salinity of the animals. These mitochondria retained their impermeability to NADH, indicating that there was no major membrane disruption. Hypertonic (but slightly hypo-osmotic) isolation media are routinely used for the isolation of mitochondria from most vertebrates (Nedergaard & Cannon, 1979), suggesting that mitochondrial shrinkage during isolation may be a critical factor minimizing mechanical damage during the isolation procedure. Isolation medium osmolarity had no effect on the osmolarity at which the maximal rate of glutamate oxidation occurred. Consequently, any differences in osmotic properties associated with glutamate oxidation between the acclimation groups can be attributed to incubation conditions, not isolation methodology.

The osmotic optimum of the mitochondria from animals acclimated to full-strength sea water is a concentration that is hypo-osmotic to the intracellular environment. The rate of oxidation of glutamate in these animals will, therefore, be low under isosmotic conditions. The osmotic optimum for the state-3 rate of oxidation of glutamate by mitochondria from animals acclimated to low salinities is a medium slightly hyperosmotic to the intracellular environment. In animals chronically exposed to dilute sea water, a higher sustained rate of amino acid oxidation may be required to maintain osmolyte concentrations at lower steady-state levels. We have suggested that osmotic effects on mitochondrial oxidation of amino acid osmolytes are involved in cell volume regulation during osmotic stress (Ballantyne & Storey, 1983, 1985; Ballantyne & Moon, 1985, 1986; Ballantyne *et al.* 1986; Moyes *et al.* 1986). This mechanism may be reduced in animals adapted to low salinity as a result in the shift in osmotic profiles during the salinity acclimation process. Animals acclimated to full-strength sea water have a greater range of osmolarities over which the mitochondria can respond adaptively to osmotic stress. This would permit a wider scope of response to changing environmental salinity.

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