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BUFFERING CAPACITIES OF THE TISSUES OF MARINE MOLLUSCS[1]

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Buffering capacities (β) (measured in slykes = micromoles of base required to titrate the pH of 1 g wet weight of tissue by one pH unit between pH 6 and pH 7) due to nonbicarbonate buffers were measured for the tissues of three marine molluscs, the oyster, *Crassostrea virginica,* the cherrystone clam, *Mercenaria mercenaria,* and the channeled whelk, *Busycotypus canaliculatum.* In general β was higher in muscle tissues (adductors, foot, heart, radular retractor) than in the gill and mantle, but hepatopancreas of the whelk had a β two- to threefold higher than that of any other whelk tissue. Selected tissues (gill of oyster, catch adductor of clam, heart and hepatopancreas of whelk) showed a significant increase in buffering capacity when tissues were isolated from animals held under anoxic conditions compared with control aerobic animals; however, no consistent effect of environmental anoxia was found, nor was buffering capacity related to the tissue levels of succinate accumulated during anaerobiosis. Tissues of oysters and clams showed a positive correlation between β and the activities of terminal glycolytic dehydrogenases, and white muscle–type tissues (adductors, foot) of all three species showed a positive correlation between β and total dehydrogenase activities (lactate, octopine, and alanopine/strombine dehydrogenases). Buffering capacity appears to be related to the tissue need for "burst" glycolytic energy production (functional anoxia) rather than to the capacity for long-term anaerobiosis (environmental anoxia).

INTRODUCTION

The pH of the cell is controlled within strict limits under most physiological situations. Intracellular pH is maintained through the buffering capabilities of several classes of cellular compounds including bicarbonate, phosphates, and imidazole compounds (e.g., protein-bound histidyl residues, histidine-containing dipeptides) (Burton 1978; Castellini and Somero 1981). Recent evidence indicates that the buffering capacity of a tissue is an adaptable parameter which can be altered to match metabolic function. Castellini and Somero (1981) have surveyed vertebrate muscles and found a strong correlation between the buffering capacity due to nonbicarbonate buffers and muscle lactate dehydrogenase activity or myoglobin content. Thus muscles (e.g., skeletal muscle of diving mammals, white muscle of fish) with a high capacity for anaerobic function and which accumulate lactic acid as a metabolic end product showed the highest buffering capacities. A high buffering capacity will allow prolonged glycolytic work with its attendant lactic acid production without the adverse effects on metabolism of a rapidly dropping intracellular pH.

The present study analyzes the buffering capacity due to nonbicarbonate buffers of the tissues of three species of marine molluscs. The oyster, *Crassostrea virginica,* and the cherrystone clam, *Mercenaria mercenaria,* are excellent facultative anaerobes which survive anoxia with an altered energy metabolism coupled to the accumulation of alanine and organic acids (succinate and/or proprionate) as metabolic end products (Collicutt and Hochachka 1977; Eberlee et al. 1983; Korycan and Storey 1983). The channeled whelk, *Busycotypus canaliculatum,* has a similar anaerobic metabolism but a lower capacity for long-term survival of anoxia (Eberlee and Storey, in preparation). Buffering capacities of the tissues of the three species were, in general, higher in muscle compared with nonmuscle tissues. Buffering capacity did not appear to be related to the anoxic accumulation of

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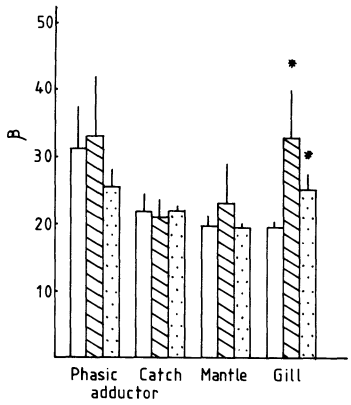

FIG. 1.—Buffering capacities due to nonbicarbonate buffers in tissues of the oyster, *Crassostrea virginica.* Buffering capacity is given in slykes: micromoles of base (NaOH) required to titrate the pH of 1 g wet weight of tissue by one pH unit between pH 6 and pH 7. Data show the average ± SEM of samples taken from three individuals under each condition. Control aerobic animals (clear) are compared with animals subjected to 96 h of anoxia stress (stripes) and with animals given 1 h of aerobic recovery from anoxia stress (dots). * = value significantly different from the control, $P < .10$.

succinate by tissues, nor was buffering capacity altered during anoxia in most tissues. In white muscle-type tissues and gill and mantle, buffering capacity was positively correlated with the activities of terminal glycolytic dehydrogenases.

MATERIAL AND METHODS

ANIMALS

Cherrystone clams, *Mercenaria mercenaria,* and oysters, *Crassostrea virginica,* were obtained from a local seafood retailer. Channeled whelks, *Busycotypus canaliculatum,* were purchased from the Marine Biological Laboratory, Woods Hole, Massachusetts. All animals were held without feeding in aerated recirculating seawater tanks at 18 C for a least 1 wk prior to use.

ANOXIA AND RECOVERY EXPERIMENTS

Control, aerobic animals were sampled directly from the seawater tanks. To impose anoxia, animals were removed from the seawater and placed in air in large jars. These were then flushed with nitrogen gas for 20 min and tightly sealed. Clams and oysters were held under anoxic conditions for 96 h and whelks for 24 h. After anoxia stress, some animals were returned to the aerated seawater tanks and were sampled during the recovery from anoxia: oysters after 1 h, whelks after 4 h, and clams after 6 h of recovery. In all cases tissues were rapidly dissected out of the animals, blotted, and immediately frozen in liquid nitrogen. Tissues were stored at −80 C until used.

MEASUREMENT OF BUFFERING CAPACITY

Tissue buffering capacities (β) due to nonbicarbonate buffering compounds were measured by the method of Bate Smith (1938) as outlined by Castellini and Somero (1981). Tissue samples (approximately 0.5 g) were homogenized 1:20 (wt/vol) in 0.9% NaCl. NaOH (0.01 N) was used to titrate the homogenate between pH values of approximately 6 and 7. A Chemtrix 60A pH analyzer with Sensorex S900C polymer-bodied electrode was used for monitoring pH changes. Titrations were performed at 23 C. Statistical tests comparing buffering capacities in aerobic versus anoxic tissues or testing the slope in linear regressions were performed using Student's *t*-test (Mendenhall 1971).

RESULTS

Buffering capacities due to nonbicarbonate buffers are shown in figures 1, 2, and 3 for the tissues of the oyster, cherrystone clam, and channeled whelk, re-

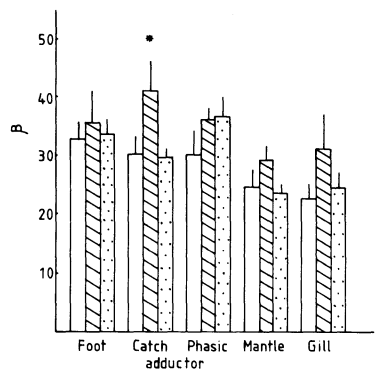

FIG. 2.—Buffering capacities due to nonbicarbonate buffers in tissues of the cherrystone clam, *Mercenaria mercenaria.* Buffering capacity is given in slykes. Data show the average ± SEM of samples taken from three individuals under each condition. Control aerobic animals (clear) are compared with animals subjected to 96 h of anoxia stress (stripes) and with animals given 6 h of aerobic recovery after anoxia stress (dots). * = value significantly different from the control, $P < .10$.

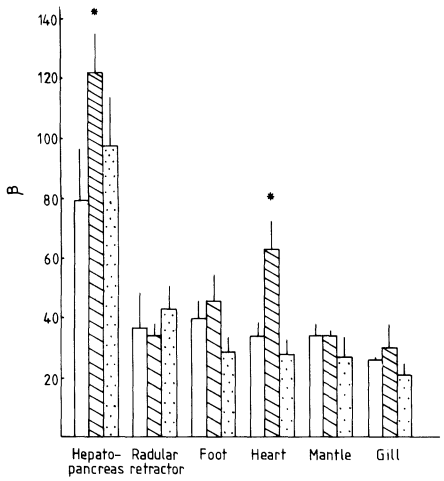

FIG. 3.—Buffering capacities due to nonbicarbonate buffers in tissues of the channeled whelk, *Busyoctypus canaliculatum*. Buffering capacity is given in slykes. Data show the average ± SEM of samples taken from three individuals under each condition. Control aerobic animals (clear) are compared with animals subjected to 24 h of anoxia stress (stripes) and with animals given 4 h of aerobic recovery after anoxia stress (dots). * = value significantly different from the control, $P < .10$.

spectively. Buffering capacity (β) is measured in slykes, the micromoles of base (NaOH) required to titrate the pH of 1 g wet weight of tissue by one pH unit over the range pH 6–pH 7 (van Slyke 1922). Tissues from control animals are compared with those taken from anoxic animals (exposed to an N_2 gas atmosphere) and with those taken from animals during recovery (aerated seawater) from anoxia. The length of anoxic exposure for the three species was chosen based on previous studies which showed >95% survival and high accumulations of anaerobic end products (succinate and alanine) after 96 h of anoxia for oysters and clams and 24 h of anoxia for whelks (Eberlee et al. 1983; Korycan and Storey 1983; Eberlee and Storey in preparation). The length of aerobic recovery was similarily chosen from these previous studies (1 h for oysters, 6 h for clams, and 4 h for whelks) and represents a time at which a large portion of accumulated acid end product (succinate) had been catabolized.

Buffering capacities of control aerobic oyster tissues (fig. 1) were lowest in gill (19.4 ± 0.80 slykes) and mantle (19.6 ± 1.1), higher in catch adductor muscle (22.2 ± 2.3), and highest in phasic adductor (30.7 ± 6.5). Anoxia had no significant effect on β in adductor muscles or mantle, nor was β significantly different from controls in the aerobic recovery period. However, in gill, buffering capacity increased by 69% during anoxia and remained significantly elevated during the recovery period.

Overall the buffering capacities of control clam tissues were higher than those of oyster tissues ranging from 22.3 ± 2.4 slykes for gill to 32.4 ± 3.1 slykes for foot muscle. Buffering capacity was higher in the muscle tissues (averaging 30.7 slykes) than in the nonmuscle tissues, gill and mantle (average = 23.4 slykes). Anoxia resulted in no significant change in β in four tissues, with only catch adductor muscle showing a significant (37%) increase in β during anoxia. Buffering capacities of tissues taken from animals during the aerobic recovery from anoxia were not significantly different from those in control animals.

Buffering capacities in tissues of the gastropod whelk were higher than those of the two bivalve species. In five tissues, β ranged from 26 ± 0.7 slykes in gill to 40 ± 5.9 slykes in foot muscle, whereas hepatopancreas showed a very high β of 79.4 ± 17.2 slykes. Again muscle tissues showed an overall buffering capacity (average = 37 slykes), higher than that of gill and mantle (average = 29.9 slykes). Anoxia resulted in a significant increase in buffering capacity in heart (84%) and hepatopancreas (55%), but, during recovery from anoxia, β in all tissues was not significantly different from that in controls.

DISCUSSION

Buffering capacities due to nonbicarbonate buffers were lower in the tissues of these marine molluscs (range = 20–40 slykes, whelk hepatopancreas excepted) than those reported for vertebrate tissues (an average of 54.7 slykes for mammalian muscle and 106 slykes for white muscle of fast-swimming fishes) (Castellini and Somero 1981). However, β of molluscan tissues was similar to that measured for crustacean muscle (England and Baldwin 1983).

Tissue specific differences in buffering capacities were found in all three species. Muscle tissues had the highest buffering ca-

pacities, with the average β of gill and mantle tissues being 74% and 81%, respectively, of those found in muscle.

Numerous cellular reactions result in the production or consumption of protons and ionized metabolites, which in the absence of adequate buffering could result in wide fluctuations in intracellular pH (Hochachka and Mommsen 1983). Anaerobic glycolysis is perhaps the major pathway generating acidic end products, and in situations such as burst muscular work produces these products at a very high rate. Not surprisingly, therefore, Castellini and Somero (1981) found that muscle buffering capacity was higher in vertebrate muscles with a high capacity for anaerobic work (as measured by lactate dehydrogenase activity). Energy production using the reactions of anaerobic glycolysis typically occurs in two situations: (1) environmental anoxia and (2) functional anoxia (tissue energy demand outstrips aerobic capacity). In vertebrates both of these situations are characterized by the accumulation of lactic acid as the end product of glycolysis. In marine molluscs, however, environmental anoxia is met by an altered metabolism culminating in the accumulation of succinate and alanine (De Zwaan 1972), whereas situations requiring added glycolytic energy output to supplement aerobic metabolism (muscle work, metabolic recovery from anoxia) result in the accumulation of imino acids (octopine, alanopine, strombine) or in some cases lactate (Zurburg, de Bont, and De Zwaan, 1982; Eberlee et al. 1983; Storey and Storey 1983).

To investigate the factors which might influence tissue buffering capacity in marine molluscs, we compared β with the measured accumulation of succinate in tissues during environmental anoxia and with the measured activities of terminal glycolytic dehydrogenases (a measure of the capacity for functional anoxia) (table 1). No

TABLE 1

COMPARISON OF THE BUFFERING CAPACITIES OF MARINE MOLLUSC TISSUES WITH THE ACTIVITIES OF CYTOSOLIC DEHYDROGENASES AND WITH THE MEASURED ACCUMULATION OF SUCCINATE AS AN END PRODUCT OF ANOXIA IN THE TISSUES

	Aerobic Buffering Capacity	Dehydrogenase Activities				Anoxic Succinate Accumulation
		ADH/SDH	ODH	LDH	Total	
Crassostrea virginica:						
Phasic adductor	30.7	19.9	0	.19	20.1	1.3
Catch adductor	22.2	5.1	0	.26	5.4	1.3
Mantle	19.6	2.6	0	.44	3.0	2.2
Gill	19.4	2.3	0	.33	2.6	1.0
Mercenaria mercenaria:						
Phasic adductor	29.8	41.0	0	.34	41.3	11.0
Catch adductor	29.8	33.0	0	.70	33.7	13.0
Mantle	24.5	8.0	0	.75	8.8	21.5
Gill	22.3	6.0	0	.83	6.8	25.0
Foot	32.4	77.0	0	2.5	79.5	15.0
Busycotypus canaliculatum:						
Foot	40.0	47.2	40.6	.4	88.2	6.5
Mantle	33.7	17.8	7.2	0	25.0	...
Heart	34.4	83.7	53.1	28.9	165.7	24.5
Radular retractor	36.7	74.5	150.6	22.5	247.6	3.5
Gill	26.0	9.6	1.3	0	10.9	4.0
Hepatopancreas	79.4	26.4	1.9	0	28.3	1.0

NOTE.—Buffering capacities are the aerobic, control levels reported in figs. 1–3. Enzyme activities of alanopine/strombine dehydrogenase, octopine dehydrogenase, and lactate dehydrogenase are expressed as μmol NADH oxidized/min · g wet weight, and the net increase in tissue succinate concentration after anoxia exposure is given in μmol/g wet weight. Enzyme activities and succinate concentrations are taken from Eberlee et al. (1983) and Korycan and Storey (1983) for oyster and clam tissues, respectively. For whelks, enzyme activities are from Plaxton and Storey (1982), and succinate concentrations are from Eberlee and Storey (in preparation).

positive correlations were found between buffering capacity of tissues and succinate production during anoxia. In *Mercenaria mercenaria,* in fact, the two parameters were inversely related. It appears, therefore, that differences in β between tissues and between species are not related to the metabolic requirements for environmental anoxia. Supporting this is the additional observation that the species with the lowest tolerance for environmental anoxia (the whelk: maximum anoxic survival approximately 24 h vs. at least 96 h for the clam and oyster) had the highest tissue buffering capacities. In addition, there was no clear effect of anoxia on tissue buffering capacity (figs. 1–3). Selected tissues of the three species showed an elevation of β during anoxia, but these included both muscle and non-muscle tissues, tissues with high or low total dehydrogenase activities, and tissues which accumulated high or low amounts of succinate during anoxia.

Although tissue buffering capacity does not appear to be positively related to the ability to survive environmental anoxia, β was correlated with the potential for glycolytic energy production, as measured by the activities of terminal glycolytic dehydrogenases. For the two bivalve species, buffering capacity was positively correlated with total cytosolic dehydrogenase activity; the equations relating β and total dehydrogenase activity (DH) were $\beta = 0.64(DH) + 18$ for oyster ($r = .996$, $P < .01$) and $\beta = 0.13(DH) + 23$ for clam tissues ($r = .92$, $P < .05$). Total cytosolic dehydrogenase activity and β were not, however, correlated for the tissues of the whelk, nor was β correlated with the individual activities of either alanopine or octopine dehydrogenase in the whelk. When white muscle tissues (adductor, foot) of the three species were considered, buffering capacity was found to be positively correlated with total dehydrogenase activity ($\beta = 0.15[DH] + 24$, $r = .86$, $P < .05$). This is similar to the positive correlation between buffering capacity and lactate dehydrogenase activity found by Castellini and Somero (1981) for the muscles of mammals and fishes. As suggested by these authors, muscle buffering capacity appears, therefore, to be related to the potential for burst glycolytic energy production. Muscle function in adductors and foot, with their low mitochondrial densities, is probably almost solely glycolytic, the intermittent and burst functioning of these muscles being supported by anaerobic carbohydrate degradation. Buffering capacity and total cytosolic dehydrogenase activity were also positively correlated in the gill and mantle tissues of the three molluscs ($\beta = 0.64(DH) + 18$, $r = .993$, $P < .01$). High rates of glycolytic energy production are encountered in these tissues during the metabolic recovery from anaerobiosis with alanopine and/or strombine accumulating (Zurburg, et al. 1982; Eberlee et al. 1983).

Overall, then, although intracellular buffering of acidic end products produced during environmental anoxia is required during anaerobiosis, the buffering capacities of tissues and species of marine molluscs do not appear to be related to the capacities for anaerobiosis. Instead buffering capacities are correlated with (and may be modified in response to) tissue capacity for glycolytic energy production.

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