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Adenylate Energy Charge and Adenine Nucleotide Measurements as Indicators of Stress in the Mussel, *Mytilus edulis*, Treated with Dredged Material under Laboratory Conditions

Gerald E. Zaroogian and Mary Johnson

U.S. Environmental Protection Agency, Environmental Research Laboratory, Narragansett, Rhode Island 02882, USA

A biochemical marker or indicator of stress such as the adenylate energy charge (AEC) (Atkinson 1977) can be used to gain information on the physiological condition of an organism prior to the occurrence of irreversible changes. An indicator such as AEC may provide an integrated estimate of effects of interactions between pollutants and environmental factors as occurs in field situations. Vetter and Hodson (1985) have commented on the utility of adenylate concentration measurements as stress indicators and among the advantages, the most obvious is the fact that these measurements can provide an instant picture of the physiological state.

Adenylate energy charge is an indication of the amount of energy available to an organism from the adenylate pool. It is calculated from measured concentrations of three adenine nucleotides, adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP), which are integral to the energy metabolism of all organisms (Atkinson 1977). The AEC, defined as (ATP + 1/2 ADP)/ (ATP + ADP + AMP), has a maximum value of 1.0 when all adenylate is in the form of ATP and a minimum value of 0 when all adenylate is in the form of AMP (Atkinson and Walton 1967). Therefore, a knowledge of the energy charge of key species with known responses to particular environmental conditions may provide a convenient measure to assess the extent to which these species are stressed. Sediment from a relatively clean site in Long Island Sound and a highly contaminated sediment from Black Rock Harbor, Connecticut which contained high concentrations of PCBs, PAHs and some metals were used to determine if any observable stressful effect, as indicated by AEC, was due to the physical action of the suspended material rather than to a toxic compound.

Accordingly, the objective of this study was to evaluate the applicability of AEC as a measure of stress in a filter feeder, the mussel Mytilus edulis, treated with dredged material under laboratory conditions and to determine the degree of variability inherent in the test.

Send reprint requests to G.E. Zaroogian at the above address

MATERIALS AND METHODS

Reference sediment (REF) for this study was collected from a reference site located in Central Long Island Sound, U.S.A. (40°7.95 N and 72°52.7 W) and was reported to contain PCBs (39 ng g^{-1}), PAHs (4500 ng g^{-1}) and metals Cu and Cr at 60 and 50 $\mu g\ g^{-1}$ dry weight respectively (Lake et al. 1988). Sediment was collected with a Smith-McIntrye grab sampler (0.1-m^2) and returned to the laboratory where it was press sieved (wet) through a 2-mm mesh stainless steel screen, homogenized in a tub with a paddle and stored in polypropylene or glass containers at 4°C until needed.

Black Rock Harbor sediment (BRH) which was collected from 25 locations within the Black Rock Harbor (Bridgeport, Conn., U.S.A.) study area with a 0.1-m^2 gravity box corer to a depth of 1.21 m contained PCBs (6400 ng g^{-1}), PAHs (142,000 ng g^{-1}) and metals Cu and Cr at 2900 and 1480 $\mu g\ g^{-1}$ dry weight respectively (Lake et al., 1988). The sediment was homogenized, distributed to barrels, and stored at 4°C. The sediment was prepared for testing as above.

Mytilus edulis (50-55mm shell length) were collected with a scallop dredge from a clean site near Dutch Island in the West Passage of Narragansett Bay, RI, U.S.A., (71°24.0 W and 41°29.4 N), from depths ranging between 5m and 10 m. The mussels were held 7 to 10 days in a laboratory flow-through system with unfiltered Narragansett Bay sea water at ambient temperature (5 to 13°C) and salinity (29 to 31 ppt). Acclimation was conducted in flowing unfiltered seawater at a rate of 1°C per day to the 15°C test temperature.

Essentially identical dosing systems, one for REF and one for BRH, provided a delivery of concentrated sediment slurry in seawater. Suspended particulates were constantly recirculated past a three-way valve. Argon gas was added to the reservoir of the dosing system to minimize oxidation of the slurry. Opening/closing of the three-way valve was controlled with a microprocessor programmed to deliver a pulse of slurry at periodic time intervals. In the mixing chamber, concentrated slurry was mixed with seawater to the proper concentration of suspended solids and distributed at a flow rate of 300 ml min^{-1} to the individual test chambers.

During the conduction of a test, REF and BRH sediments were maintained in an argon atmosphere to prevent oxidation prior to being pumped into the mixing chamber of the dosing system. The treatment combinations were obtained by proportionally siphoning suspended sediment from the appropriate distribution chambers to produce a combined flow of 300 ml/min in each exposure chamber.

Forty M. edulis were continuously fed laboratory cultured Isochrysis galbana at a rate of 94 mg (dry weight) per mussel per day. Conditions and techniques of algal culture were modified after Guillard (1975). Guillard's "f/2" nutrient media was used, except that all trace metals but iron were eliminated and the

concentration of the vitamins, thiamin and B12 were doubled.

On day 0 and 28, M. edulis were sampled for AEC. Test I was terminated on day 26 instead of day 28 due to a significant reduction in feeding by mussels in the 100% BRH treatment at this time. This reduced feeding was interpreted as a rapidly deteriorating health condition which could have resulted in death of mussels prior to 28 days. Therefore, it was thought prudent to terminate at 26 days.

Within 10 min of removal from the dosing system, the adductor muscle was rapidly dissected out,[1] blotted dry, placed on a labelled polythene strip (Gladwrap[1]) and freeze clamped with aluminum blocks cooled to -196°C with liquid nitrogen (Ivanovici 1980; Bergmeyer 1965).

Adenine nucleotides were extracted from tissues using the method of Zaroogian et al (1982) and the concentrations of ATP, ADP, and AMP were determined spectrophotometrically (340 nm) with hexokinase (Lamprecht and Trautschold 1974), pyruvate kinase and myokinase (Adam 1963), respectively. All enzymes, chemicals and reagents (analytical grade) were obtained from Boehringer Mannheim, Indianapolis, Indiana.

Means and standard error within a treatment for tests I and II were calculated for the concentrations of the individual adenine nucleotides and the AEC. The data were analyzed by analysis of variance (ANOVA) to detect differences among treatments and to determine the reproducibility of AEC among treatments within a test. Fisher's Least Significant Difference was used to make pairwise comparison of means between treatments (Snedecor and Cochran 1980).

RESULTS AND DISCUSSION

Survival of M. edulis was 100% in all treatments except for the 100% BRH (97%) and 50% BRH (87%) treatments in test I. Analyses of variance indicated that the AEC for the treatment 50% REF/50% BRH was significantly lower from all other treatments (Table 1). Estimated mean AEC values decreased over the exposure period (Table 1). However, only those from mussels treated with 50% REF/50% BRH were significantly ($\alpha < 0.05$) different from those AEC values obtained at the start of treatment and controls (Table 1). The ATP/ADP ratios were lowest in those mussels exposed to 50% REF/50% BRH. In comparison, the adenylate pool was lowest in animals exposed to 100% BRH sediment (Table 1). Pool size in Test II mussels at Time 0 was the smallest of all treatments; however, due to variability, no significant differences were obtained between adenylate pool size and treatments. Except for Time 0, adenylate pool size was larger in test II mussels than in test I

[1] Registered trademark. Mention of trade names of commercial products does not constitute endorsement or recommendation for use.

Table 1. The response of adenine nucleotides in M. edulis adductor muscle tissue after treatments with BRH sediment for 26 days (test I) and 28 days (test II) under laboratory conditions.

Treatment	n*	ATP	μ mol/g Wet Weight Tissue ADP	AMP	ATP+ADP+AMP	AEC	
			Test I				
Time 0**	9	3.63(0.16)***	0.93(0.05)***	0.14(0.01)***	4.70(0.18)	0.87(0.01)***	A****
100% REF	10	2.86(0.09)	1.07(0.08)	0.16(0.03)	4.09(0.16)	0.83(0.01)	A
100% BRH	10	2.70(0.13)	0.97(0.06)	0.16(0.04)	3.83(0.19)	0.83(0.01)	A
50% REF/50% BRH	10	2.38(0.11)	1.22(0.04)	0.36(0.04)	3.96(0.12)	0.75(0.01)	B
			Test II				
Time 0 **	10	3.35(0.19)***	0.93(0.05)***	0.08(0.01)***	4.36(0.21)	0.87(0.01)***	A****
100% REF	10	3.53(0.15)	1.21(0.07)	0.19(0.03)	4.93(0.21)	0.83(0.01)	A
100% BRH	10	3.36(0.19)	1.23(0.08)	0.17(0.03)	4.76(0.26)	0.83(0.01)	A
50% REF/50% BRH	10	3.30(0.21)	1.35(0.03)	0.25(0.04)	4.90(0.21)	0.80(0.01)	B

* n = Number of mussels sampled per treatment.
** Start of treatment.
*** Mean value of each sample with standard error of mean in parentheses.
**** Means with different letters differ significantly at $\alpha \leq 0.05$.

mussels from all treatments (Table 1). Changes in the adenylate pool were not consistent with lower AEC values (Table 1).

The comparative differences observed in the AEC values after 26 days of treatment between 50% BRH/50% REF sediment and 100% BRH sediment seemed consistent with observations reported by Giesy et al. (1983). Since no observable effects were obtained with reference sediment, it would appear that the physical action of the suspended material does not elicit a response. Although it appeared that the significantly lower AEC found with the 50% BRH/50% REF treatment was due to the toxic effect of chemical compounds, the relationship was not clear since a significantly lower AEC was not obtained with 100% BRH. Lake et al. (1984) reported that BRH dredged material was highly contaminated with PCB's, PAHs, Cu and Cr. In the same report, bioaccumulation studies with M. edulis indicated that 44% of the sediment PCBs, 28% of the sediment PAHs and various amounts of the Cu and Cr accumulated in the soft tissues during a 28-day exposure to BRH dredged material. Thus, it is likely that the same materials were accumulated by M. Edulis during this experiment. Mytilus edulis is able to undergo anaerobic respiration for long periods of time (5 to 7 days) (Wijsman, 1976). Shell closure and anaerobiosis may enable M. edulis to avoid continuous ingestion of toxicants which they are able to sense. The lower BRH sediment concentration used in this study may have contained contaminants whose concentrations were below the threshold concentration at which the mussels closed to avoid exposure. It would be unlikely that the mussels treated with 100% BRH closed their shells and survived anaerobically for 26 days. However, it is possible that these mussels opened their shells only intermittently for feeding and excretion. Nelson et al.(1985) reported that mussels treated with 100% BRH exhibited a significantly lower clearance rate when compared to other treatments. In contrast to the lower clearance rates, Nelson et al. (1985) reported no differences in respiration rates in M. edulis among treatments with BRH dredged material. However, during acute tests, Brown and Newell (1972) observed a decrease in whole animal respiration rates in M. edulis during exposure to Cu. Scott and Major (1972) reported a similar decrease in the same species. It is evident from the literature that no one respiration rate response is elicited consistently after exposure to a pollutant.

The reproducibility between tests was acceptable with identical AEC values for the same treatment between tests except for the 50% REF/50% BRH treatment which differed significantly. In addition, 13% mortality was recorded for the 50% REF/50% BRH treatment in test I as opposed to no mortality for the same treatment in test II. These facts also helped to explain the lower AEC value (0.75) obtained with mussels from the 50% REF/50% BRH treatment in test I when compared to the AEC value (0.80) obtained with mussels from the same treatment in test II. Although the data would suggest that improvement in reproducibility of AEC measurement for the 50% REF/50% BRH treatment is wanting, it appeared that the mussels in the 50% REF/50% BRH treatment from test II were in better health

condition and more active metabolically than their counterparts in test I as indicated by the AEC after 26 days.

The quantitative relationship between statistical significance and biological significance has to be refined. Vetter and Hodson (1982) reported that changes in AEC were always accompanied by changes in total adenylate concentration. Our results support this observation; however, the changes did not always parallel each other, as indicated in test II, where one of the highest pool concentrations was associated with the treatment having the lowest AEC.

Atkinson (1977) reported that stress conditions responsible for a decrease in AEC usually cause a decrease in size of the adenylate pool, sometimes by a much larger factor. Ordinary metabolic uses of ATP generate equimolar amounts of ADP or AMP so that no change in adenylate pool size would be expected. However, a decrease in the total adenylate concentration could result from deamination of AMP by adenylate deaminase which would raise the AEC and ATP/ADP ratio. This did not appear to be the situation in this study since the pool size did not decrease with a corresponding decrease in ADP. After mummichog exposure to kraft mill effluent at high dissolved oxygen concentrations, Vetter et al.(1986) reported consistent changes between AEC and total adenylates. However, at low dissolved oxygen, changes between AEC and total adenylate concentrations were not consistent. During acclimatization to 15° C in unfiltered seawater, increased filter-feeding activity of mussels would consume much energy. Further, increased activity of digestion and excretion should also result in an energy demand. Mussels in March would probably have minimum nutrient reserves; therefore,energy would be acquired primarily from feeding activity, and low food concentration could result in reduced energy reserves. Winter (1975) reported that M. edulis in the presence of small amounts of food were stimulated to open their shells and actively feed; however, the energy consumed was higher than the energy gained. This could explain why the adenylate pool at Time 0 in test II was low and increased during treatment when food supply was adequate.

Vetter and Hodson (1985) have suggested that a combination of adenylate measurements with other measures such as glycogen may improve detection of low-level chronic stress. As a test of this thesis, Vetter et al. (1986) studied adenine nucleotide and glycogen concentrations concurrently in white muscle of mummichogs after exposure to kraft mill effluent. At high dissolved oxygen, the AMP and glycogen concentrations decreased significantly whereas ATP, ADP, total adenylate and AEC remained unchanged. Perhaps adenine nucleotide measurements in conjunction with glycogen may have merit as a more sensitive indicator of sublethal stress. However, we abandoned the use of glycogen in our suite of measurements since seasonal and individual variability in bivalve molluscs were exceptionally high.

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Environmental Protection Agency, Environmental Research Laboratory, South Ferry Road, Narragansett, RI 02882

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