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Application of adenylate energy charge to problems of environmental impact assessment in aquatic organisms

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ABSTRACT: Various physiological and biochemical methods have been proposed for assessing the effects of environmental perturbation on aquatic organisms. The success of these methods as diagnostic tools has, however, been limited. This paper proposes that adenylate energy charge overcomes some of these limitations. The adenylate energy charge (AEC) is calculated from concentrations of adenine nucleotides ([ATP + ½ADP]/[ATP + ADP + AMP]), and is a reflection of metabolic potential available to an organism. Several features of this method are: correlation of specific values with physiological condition or growth state, a defined range of values, fast response times and high precision. Several examples from laboratory and field experiments are given to demonstrate these features. The test organisms used (mollusc species) were exposed to a variety of environmental perturbations, including salinity reduction, hydrocarbons and low doses of heavy metal. The studies performed indicate that the energy charge may be a useful measure in the assessment of environmental impact. Its use is restricted, however, as several limitations exist which need to be fully evaluated. Further work relating values to population characteristics of multicellular organisms needs to be completed before the method can become a predictive tool for management.

INTRODUCTION

Many recent studies have examined the potential of various physiological and biochemical methods for assessing an organism's state of well-being in perturbed and non-perturbed aquatic environments (Jeffries, 1964, 1972; Lynch, 1974; Davis, 1977; McLeay & Howard, 1977; McIntyre et al., 1978). Several problems limit the application of many of these methods to environmental monitoring programmes (Sprague, 1971; McErlean et al., 1972; Swartz, 1972; Waldichuk, 1973). The problems include: specificity of response to a species or phyletic group; differences between species; lack of ecological relevance; seasonal variation; low levels of precision, which lead to high levels of variability of response and which subsequently require large sample sizes ($n \geqq 20$ organisms) for statistically detectable differences; difficulty of objective assessment without complex and expensive methodology; lack of consistent responses; slow response time; unsuitable for application in the field and the laboratory. Despite awareness of these problems since the late 1960s, the need for a technique(s) which overcomes at least some of these problems is still felt (McLeay & Howard, 1977).

A biochemical measure known as adenylate energy charge (AEC) has been recently shown to overcome some of the above problems (Ivanovici, 1977a, b, 1980a; Giesy et al., 1978). Furthermore, it has been proposed as a method which has great potential for

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evaluation of the responses of organisms to environmental perturbations (Ivanovici, 1974; Wiebe & Bancroft, 1975; Ivanovici & Wiebe, 1980), especially in a monitoring programme.

In this presentation I shall describe several examples from aquatic invertebrates which illustrate some of the features of the AEC response. I should emphasise, however, that this method also has several problems which need to be properly evaluated before its potential as a monitoring and predictive tool can be verified.

DEFINITION AND BASIC RESPONSE OF ADENYLATE ENERGY CHARGE

The AEC is defined as the metabolic energy potentially available from the adenylate pool to an organism at the time of sampling (Atkinson & Walton, 1967; Atkinson, 1968). It is calculated from measured concentrations of adenosine-5′-triphosphate (ATP), -diphosphate (ADP) and -monophosphate (AMP) by the formula: (ATP + ½ADP)/(ATP + ADP + AMP). By definition values of AEC range from 0 to 1.0.

From theoretical and empirical considerations, Atkinson (1968) predicted that values of AEC would be between 0.8 to 0.9 in all organisms under physiologically optimal conditions. This was initially verified by Chapman et al. (1971), who also showed that values of AEC below 0.8 occurred when conditions became limiting or sub-optimal, and that organisms whose AEC decreased below 0.50 did not recover when returned to optimal conditions. Growth and reproductive rates were much reduced with values below 0.8. Montague & Dawes (1974), Wiebe & Bancroft (1975) and Reece et al. (1976) found similar responses in a variety of microorganisms. The AECs of numerous multicellular organisms have been shown to respond in a similar fashion (e. g. vertebrates: Ridge, 1972, Thillart et al., 1976, Chulavatnatol & Haesungcharern, 1977; plants: Ching et al., 1975, Bewley & Gwozdz, 1975; invertebrates: Ivanovici, 1974, Behm & Bryant, 1975, Wijsman, 1976), although the relationship between changes in AEC and effects on growth and reproductive potential are not well documented.

RESPONSES OF ADENYLATE ENERGY CHARGE UNDER LABORATORY CONDITIONS

Table 1 shows a typical response of AEC in the estuarine gastropod *Pyrazus ebeninus* to reduction in salinity. Decreases in salinity below 20 ‰ were reflected by significant reductions in AEC within 24 h. Further reductions did not occur unless the exposure period was extended past 7 days (see Ivanovici, 1980a). Although the response to salinity was not affected at 29 °C, values in all groups were approximately 20 % lower than at 20 °C. When molluscs were returned to control conditions of 34 ‰ at 20 °C from the reduced salinities at both temperatures, the only molluscs which did not recover were those whose AEC decreased by 40 % to approximately 0.5 within 24 h (i. e. those kept at 0 and 17 ‰ at 29 °C). The adenylate pool showed no significant changes associated with variations of salinity or temperature in this and other examples, indicating its insensitivity to variations in environmental conditions (Table 1).

The data from this example illustrate a number of features. The AEC has a high level of precision. This is indicated both by the adequacy of the small sample size (3 molluscs) to detect differences that were statistically significant, and the low overall standard error

Table 1. *Pyrazus ebeninus.* Response of adenylate energy charge and total adenine nucleotides to reduction in salinity and elevated temperature (summarized from Ivanovici, 1980a). Gastropods were transferred to the indicated conditions after 1 week's acclimation to laboratory conditions. Molluscs were removed for analysis of nucleotides after exposure to the experimental conditions for 1 and 4 days. Collumela muscle was dissected from each mollusc and freeze-clamped in less than 60 s. Each freeze-clamped tissue was then pulverized (at −180 °C), 6 % perchloric acid added, pulverized further and mixed thoroughly, then allowed to thaw on ice (tissue : acid was 1:10). Each sample was centrifuged (600 g, 40 min, 0 °C), neutralized (5N K_2CO_3), then assayed for ATP, ADP and AMP. Modified spectrophotometric methods of Jaworek et al. (1974) and Lamprecht & Trautschold (1974) were used to assay ATP, ADP and AMP with hexokinase, pyruvate kinase and myokinase respectively. (Details of these methods are in Ivanovici 1977a and 1980b.) The experimental data were analysed by analysis of variance, and differences between means were tested for significance by Student-Newman-Keuls test (see Ivanovici, 1980a for details)

Salinity (‰)	Temperature (°C)	Time of sample (days)	Adenylate energy charge*	(ATP+ADP+AMP) μmol g^{-1} wet weight
0	20°	1	0.63 ± 0.07	5.74 ± 0.07
		4	0.66 ± 0.02	3.42 ± 0.24
	29°	1	0.51 ± 0.01	5.32 ± 0.45
		4	0.54 ± 0.03	5.15 ± 0.25
17	20°	1	0.66 ± 0.03	6.00 ± 0.25
		4	0.66 ± 0.02	3.81 ± 0.22
	29°	1	0.55 ± 0.01	4.96 ± 0.30
		4	0.60 ± 0.04	5.02 ± 1.31
34	20°	1	0.86 ± 0.02	5.42 ± 0.18
		4	0.89 ± 0.02	5.40 ± 0.04
	29°	1	0.73 ± 0.05	5.82 ± 0.34
		4	0.70 ± 0.01	4.52 ± 0.48

* Values are means of 3 animals ± standard error

of ± 0.034. Comparisons of the overall coefficients of variation of AEC with those of the individual nucleotides and the ratio of ATP: ADP (from data of 5 experiments, Ivanovici, 1980a) also indicate that those of the AEC have the highest level of precision. The values are: AEC, 7.4 %; ATP, 18.7 %; ADP, 19.7 %; AMP, 41.4 %; adenylate pool, 13 %; ATP : ADP, 39.9 %. The response time is rapid, occurring within 24 h. The response occurs faster and clearer than mortality data (Ivanovici, 1980a). The response of AEC to salinity and temperature in the laboratory has some ecological relevance because it lends insight into this species' distribution in the environment. For example, the species is found only in the temperate region of eastern Australia. Within the estuary, it is found only in the more saline areas, where salinity at high tide is between 30–34 ‰. Additional field studies are, however, necessary to validate this.

Studies with several other aquatic species (both estuarine and freshwater) under laboratory conditions indicate that AEC responds consistently (by reduction below normal values) when conditions deteriorate from optimal. Wijsman (1976) showed that AEC decreases in *Mytilus edulis* under anaerobic conditions. Ivanovici (in preparation) demonstrated a similar response to anaerobic conditions in another mussel species, *Trichomya hirsuta.* The response of AEC to reduced salinity has also been demonstrated

Table 2. *Pyrazus ebeninus*. Adenylate energy charge and total nucleotides of individuals sampled in the field: non-polluted environments. Values are means of *n* animals (indicated in brackets) ± S.E. Methods as described in Table 1. Molluscs freeze-clamped in the field

Sample	AEC		(ATP + ADP + AMP) μmol g^{-1} wet tissue weight		Reference
	High tide	Low tide	High tide	Low tide	
Population from Jervis Bay					
Normal conditions:					
July 1975 (5)	0.90	0.79	5.12	4.41	Ivanovici (1977a, b, in preparation)
Oct. 1975 (10)	0.86	0.80	5.86	5.23	Ivanovici (1977a, b, in preparation)
July 1976 (5)	0.87	0.80	5.64	5.46	Ivanovici (1977a, b, in preparation)
	± 0.014		± 0.22		
Abnormal conditions:					
Flood (12)	0.65 ± 0.034		5.70 ± 0.23		Ivanovici (1977a, b)
Population from Port Hacking (6)	0.85 ± 0.02		5.53 ± 0.34		Rainer et al. (1979)

in the parasitic worm, *Monieza expansa* (Behm & Bryant, 1975), as well as in two bivalve species, *Anadara trapezia* and *Saccostrea commercialis* (Rainer et al., 1979). Although the latter study demonstrated consistency of response of AEC to the stress of reduced salinity, two problems were highlighted. The AEC of *S. commercialis* was significantly lower than normal in the control groups (0.76–0.64), indicating that perhaps some species may be unsuitable for monitoring. The AEC response did not differentiate species according to their sensitivity to salinity reduction, suggesting that its usefulness may be more as a general indicator of stress – within species rather than between species. Further studies are needed here, however.

Acute and chronic exposure of *Corbicula fluminea, Anodonta imbecillis, Procambarus pubescens* and *Palaemonetes paludosos* to Cd resulted in significant reductions of AEC (Giesy et al., 1978). The response occurred rapidly (e. g. significant reduction in *C. fluminea* after only 6 h exposure to as little as 5 $\mu g\ l^{-1}$ Cd), well in advance of physiological and behavioural changes, and demonstrated high levels of precision, with sample sizes of only 5 animals. Reduced AEC levels occurred after exposure of the marine isopod *Cirolana borealis* to anoxia and 0.14 mM toluene (Skjoldal & Bakke, 1978). The lack of statistical information in this study, however, makes its interpretation more difficult.

FIELD STUDIES

The suitability of a method for the assessment of environmental perturbation must be evaluated under field as well as laboratory conditions. Factors which should be considered include: ease of sampling in the field; whether levels of precision are comparable to those under laboratory conditions; freedom from seasonal and spatial effects; and correlation with the presence or absence of stress.

The studies of Atkinson (1968) and Chapman et al. (1971) indicate that AECs of organisms in their normal environments should range between 0.8 and 0.9. Levels of AEC found in several aquatic invertebrate species sampled from normal field conditions have verified this. Furthermore, the AEC appears to be independent of seasonal, tidal and spatial differences. For example, the AECs of *Pyrazus ebeninus* sampled at different times of the year were similar (Table 2). Even though the differences between high and low tides were significant, the values were within the normal range (Table 2). AECs of *P. ebeninus* from different areas are also comparable. Similarly, Giesy et al. (1978) found high and comparable AECs in separate populations of *A. imbecillis* and *C. fluminea*. Seasonal variation of AEC has been noted, however, in several species of zooplankton (Skjoldal & Bamstedt, 1977). The levels of precision in this latter study are not as good as those described above. This suggests that methodological difficulties may contribute a large but unknown amount to the observed variability. Methodological difficulties may also account for the low AECs (0.66 ± 0.06) measured in field populations of *Saccostrea commercialis* by Rainer et al. (1979).

Lower-than-normal values of AEC have been generally consistent with the onset of suboptimal conditions in the field. Natural, but unusually large perturbations in the estuarine environment resulting in, for example, prolonged exposure to reduced salinity caused by flooding are reflected by AECs of 0.65 ± 0.034 in *Pyrazus ebeninus* (Table 2).

Molluscs subjected to man-made perturbations in the field also show significant

Table 3. *Pyrazus ebeninus.* Response of adenylate energy charge and nucleotide pool to changes in environment (summarized from Ivanovici 1977a, b, in preparation). Individuals were caged at clean and polluted sites for 14 days, returned to Moona Moona Creek and sampled after a further 14 days. Values are means of 6 animals. Methods as described in legend of Table 1

Measure	Time (days)	Controls		Experimentals	
		Moona Moona Creek	Currambene Creek	Morrisons Bay	Duck River
AEC	14	0.82	0.80	0.62	0.69
	28	0.81	0.77	0.79	0.80
ATP + ADP + AMP (μmoles g^{-1} wet weight)	14	5.99	6.40	4.52	4.48
	28	6.49	6.71	6.10	5.96

Table 4. *Trichomya hirsuta.* Adenylate energy charge and nucleotide pool in populations from the inlet and outlet sides of the Vales Point Power Station (from Ivanovici, 1977a, b, in preparation). Values are means of 6 animals. Nucleotides were extracted from adductor muscle. Methods as in legend of Table 1

Measure	Inlet		Outlet
AEC	0.78	± 0.022	0.68
ATP + ADP + AMP (μmol g^{-1} wet weight)	2.45	± 0.087	2.34
Salinity (‰)	30.7		31.0
Temperature (°C)	21.2		28.9

reductions in AEC within 24 h (Ivanovici, in preparation). For example, the AECs of *P. ebeninus* transferred to various sites in the field were depressed only in those molluscs which were transferred to sites known to be contaminated with hydrocarbons (Table 3). Values within the normal range were measured only in molluscs caged at control sites or in molluscs returned to control conditions from perturbed ones. Furthermore live molluscs were not found at the contaminated sites several months after the conclusion of the experiments. This observation associates low values of AEC with low survival, although it is not conclusive proof, and does not indicate mechanisms of action. Adenylate pool size did not change significantly under natural or man-made perturbations (Tables 2 and 3).

AECs in populations of *Trichomya hirsuta* from the inlet and outlet sides of a power plant indicate that the lower AEC was found only in the mussels from the outlet side where the environment was perturbed by fast flowing water heated 8 °C above ambient (Table 4). This low value was associated with a very sparse population, and lends indirect support for the association of low reproductive or survival potential with reduced AECs.

These field studies demonstrate the following features: (a) The methods described by Ivanovici (1977a, 1980b) enable samples to be collected easily in the field. (b) AEC has levels of precision under field conditions that are comparable with those in the

laboratory (e.g. overall S. E.s of data in Tables 3 and 4 were ± 0.029 and ± 0.022, respectively). (c) The coefficient of variation (C.V.) for AEC is the smallest of the nucleotide variables, even under field conditions, indicating its superior level of precision. For example, C.V.s from 4 experiments by Ivanovici (1977a) were also as follows: AEC, 9.7 %; ATP, 22 %; ADP, 22.1 %; AMP, 49.7 %; adenylate pool, 15.0 %; ATP:ADP, 33.7 % (Ivanovici, in preparation). These estimates reflect variation between individuals of a given sample population, and include variability due to sampling and analytical steps. Similar high levels of precision for AEC, as compared with other nucleotide variables, are found in freshwater invertebrates (C.V.s between 2 and 7 % for AEC, greater than for other nucleotide variables but not reported; Giesy et al., 1978), foetal rats (Ballard, 1971), plants (Ching et al., 1975) and aquatic microbial communities (within-sample C.V. of 8 % for AEC, compared with > 25 % for other nucleotide measurements; Witzel, 1979). (d) Seasonal and spatial variability are minimal. (e) The reduced AECs correlate with environments that can be considered to place the organisms under stress, and presumably under some reproductive disadvantage.

These studies now need to be extended to include a much greater variety of species, and to examine reproductive potential in adults and survivorship in offspring with respect to lowered AECs.

LIMITATIONS OF ADENYLATE ENERGY CHARGE

Although the data discussed above indicate that the AEC has a number of advantages over other methods, there are several limitations which indicate that its use must be approached cautiously. Since these limitations have been described in detail elsewhere (Ivanovici, 1980c; Ivanovici & Wiebe, 1980), they will only be briefly listed here:

(a) Adequate measures to either avoid or estimate the extent of breakdown of ATP during dissection, extraction and analysis are necessary to ensure in vivo estimates. Problems in methodology are a recognized source of lower-than-normal values (Knowles, 1977; Skjoldal & Bakke, 1978); current methods are laborious, time consuming and require specialized personnel. This latter aspect needs to be considered carefully in determining the size of the sampling programme.

(b) Exceptions in pattern of AEC response in perturbed environments indicate that species may need to be selected carefully, and that interspecies or interphyletic comparisons may be limited: values of AEC less than 0.8–0.9 have been measured in "normal" organisms (e. g. Zs.-Nagy & Ermini, 1972; Eigener, 1975), in some cases, despite optimization of methods (e. g. Rainer et al., 1979); variation of AEC occurs in different organs of some organisms (Wijsman, 1976); high AECs have been measured in organisms known to be moribund and to be under extremely stressful conditions (e. g. Chapman & Atkinson, 1973; Ivanovici, unpublished).

(c) Lack of predictive power of AEC measurement. Insufficient studies have been done with multicellular organisms relating the effects of reduced AEC on growth, reproductive potential and the viability of offspring of stressed adults. Until these are carried out, predictions based on lowered AECs can only remain tentative.

(d) AEC cannot identify the cause of perturbation – it can only be used as a general indicator of stress. It does not appear to differentiate between sensitive and less-sensitive species (Rainer et al., 1979).

CONCLUSION

A number of examples have been presented to illustrate that measurements of AEC do offer a number of features which indicate great potential for application to monitoring programmes of various sorts. Such programmes might include aquaculture (where optimal growth conditions need to be evaluated), monitoring for effluent effects on selected species in the environment and evaluation of new compounds and their effects on organisms in laboratory conditions. At a more basic level, AEC could lead to better understanding of distributions of species in their natural environment, since it indicates the level of metabolic potential available. The features that AEC measurement have include: consistent response to stress (perturbed environmental conditions), under laboratory and field conditions; high levels of precision both in the laboratory and in the field, resulting in the need for small sample sizes; ease of sampling; independence from spatial and possibly seasonal effects.

It was once hoped that AEC might provide the all-encompassing method that would supply the answers needed in effects-assessment (Ivanovici, 1974, 1977a, b). The available data indicate that AEC cannot be used alone as an effective predictive tool. However, its use as a general indicator of stress is not precluded, as long as the limitations are recognized, and it is measured in conjunction with at least several physiological, biochemical or other measures. Further studies are recommended.

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