Copyright ©

Kieler Meeresforsch., Sonderh. 5, 566 – 569. Kiel 1981

Maximal enzyme activities in certain polychaetes in relation to their behaviour and ecology

J. Blackstock and T.H. Pearson

Dunstaffnage Marine Research Laboratory, Oban, Argyll U.K.

Introduction

In various sea lochs in the west of Scotland characteristic successional changes in population densities and species diversity of marine polychaete worms have been related to effects of inputs of organic material from industrial installations. In their review PEARSON and ROSENBERG (1978) confirm that the opportunistic polychaetes *Capitella capitata* (Fabricius) and *Scolelepis fuliginosa* (Claparède) are both capable of rapid population growth in areas which are most affected by the organic inputs and where, as a consequence, conditions are frequently anoxic at the sediment-water interface. *Glycera alba* (Müller) is a predatory polychaete which is not found in the most affected areas and is therefore considered to be more sensitive to effects of the organic inputs than *Capitella capitata* or *Scolelepis fuliginosa*. *Melinna palmata* (Grube) has a similar spatial distribution in organic pollution gradients to *Glycera alba* and is therefore also considered to be sensitive to effects of organic enrichment.

In addition to their varied sensitivity to effects of the organic inputs the four species exhibit different behavioural characteristics. *Glycera alba* is an intermittently active carnivore and is thought to utilise short but rapid bursts of activity for capture of prey organisms. *Scolelepis fuliginosa*, a burrow dwelling surface deposit feeder, exhibits very rapid escape withdrawal reactions to disturbance and has been observed to maintain rapid movement when removed from the sediment, and *Capitella capitata* and *Melinna palmata* are less active deposit feeding tube dwelling worms.

Investigations by ZAMMIT and NEWSHOLME (1976) have indicated that maximum activities of certain enzymes associated with energy yielding metabolism in muscles of marine invertebrates may reflect relative capability for bursts of activity and the relative abilities of the invertebrates to utilise anaerobic metabolism for survial of periods of anoxia. In areas where the population densities of each of the species are increased as a consequence of organic enrichment they are significant components of the diets of pelagic and epibenthic fauna of commercial importance (FEDER, pers. communication). Investigations of the fitness of each species for survival in areas which are variously affected by organic enrichment are therefore relevant to studies of production and a preliminary study of maximal activities of some relevant enzymes in *Capitella capitata*, *Scolelepis fuliginosa*, *Glycera alba* and *Melinna palmata* has therefore been undertaken with the objective of comparative assessment of the enzyme activity 'profiles' in relation to the ecology and behaviour of each of the species.

Materials and methods

The specimens were collected from a number of appropriate locations in Loch Eil, which receives an organically rich effluent from a wood pulp mill, in Loch Creran,

which receives an input of organic effluent from a seaweed processing factory, and in the Firth of Clyde in the vicinity of an area used for disposal of solid waste from domestic and industrial sources.

Some 150 *Glycera alba*, 100 *Capitella capitata*, 60 *Melinna palmata* and 20 *Scolelepis fuliginosa* have been extracted and assayed for activities of phosphofructokinase (E.C. 2.7.1.1.), pyruvate kinase (E.C. 2.7.1.40), malate dehydrogenase – NAD dependent (E.C. 1,1,1,37), citrate synthase (E.C. 4.1.3.7.) and aspartate aminotransferase (E.C. 2.6.1.1.). Preliminary investigations have shown that improved recoveries of *Capitella capitata* pyruvate kinase and malate dehydrogenase activities were obtained in crude extracts prepared using a solution containing 800 mM-sucrose, 1 mM-dithiothreitol and 0.1 % v/v Triton X-100 (GOULD, pers. comm.). For the remaining analyses extracts of individual worms in 1–3 ml of 0.1 M-phosphate buffer were prepared as described by BLACKSTOCK (1980).

Activities of phosphofructokinase, pyruvate kinase, malate dehydrogenase and citrate synthase were estimated by the spectrophotometric methods used by BLACKSTOCK (1980) and aspartate aminotransferase activity was assayed utilising reagents, from a test kit [Boehringer Corporation (London) Ltd.], prepared to provide an assay medium containing 87 mM-phosphate buffer, pH 7.4, 34.7 mM-aspartate, 0.17 mM-NADH and 0.1 ml of suitably diluted extract. The reaction was started by adding α-oxoglutarate (7.5 mM). Control values were obtained by monitoring the decrease in A_{340} prior to the addition of α-oxoglutarate. For all assays the reaction temperature was 25°C. Enzyme activities were calculated in milli-Units (mU) $\cdot$ mg^{-1} (soluble protein in the extract) where 1 mU represents the conversion of 1 nanomole of substrate $\cdot$ min^{-1} under the conditions selected for the assays. 'Maximal' enzyme activities were calculated as the mean values of the six highest activities of each enzyme in extracts of each of the species.

Results and discussion

Malate dehydrogenase activities were of similar order in all 4 species and are relatively high (1000–1500 mU/mg protein) when compared with the activities of phosphofructokinase, pyruvate kinase, citrate synthase and aspartate amino transferase. In organisms which utilise the 'succinate' pathway of anaerobic catabolism a high malate dehydrogenase activity may prevent accumulation of oxaloacetate and thereby facilitate succinate formation via malate and fumarate (DE ZWAAN 1977). The observed maximum activities of malate dehydrogenase may therefore be indicative of a capability for anaeobic 'succinate' production in all 4 species.

For the purpose of comparison of maximal enzyme activities in each of the species the results have been expressed as % of mean maximum malate dehydrogenase activity as shown in Table 1.

Table 1

Mean relative 'maximal' enzyme activities in *Capitella capitata*, *Scololepis fuliginosa*, *Glycera alba* and *Melinna palmata*. The results are expressed as % of maximum malate dehydrogenase activity.

Enzyme	*C. capitata*	*S. fuliginosa*	*G. alba*	*M. palmata*
Phosphofructokinase	1.3	6.5	6.5	0.7
Pyruvate kinase	4.5	5.2	68.8	4.6
Citrate synthase	4.6	0.8	2.8	4.1
Aspartate aminotransferase	80.0	56.7	10.2	18.0

In *Glycera alba* the relatively high activities of phosphofructokinase and pyruvate kinase and low citrate synthase activity are typical of muscles which primarily utilise anaerobic glycolysis for energy production and are capable of short but rapid bursts of activity (ZAMMIT and NEWSHOLME 1976; ALP et al. 1976), which would be expected of *Glycera alba* in the capture of prey. Similarly the relatively high phosphofructokinase and low citrate synthase activities in *Scolelepis fuliginosa* may also relate to a relatively high capability for muscular activity which is exhibited by this species. However, in *Scolelepis fuliginosa* pyruvate kinase activities are considerably lower than in *Glycera alba*. ZAMMIT et al. (1978) suggested that low pyruvate kinase: phosphofructokinase ratios were typical of muscles which preferentially utilised the 'succinate' pathway for energy production, a process which may be of considerable energetic advantage to *Scolelepis* which is found in large numbers in oxygen deficient sediments.

In *Capitella capitata* and *Melinna palmata* relatively low activities of the glycolytic enzymes (phosphofructokinase and pyruvate kinase) may reflect a low capability for rapid bursts of activity by these tube dwelling worms. In these species citrate synthase is somewhat higher, possibly indicating a higher capability for lipid catabolism than in *Scolelepis* or *Glycera*. However, the citrate synthase activities in all 4 species remain characteristic of ''low'' citrate synthase groups examined by ALP et al. (1976) and the results are therefore consistent with a low rate of energy utilisation by *Capitella* and *Melinna*.

Capitella capitata and *Scolelepis fuliginosa* both exhibit opportunistic reproductive strategies and are found in high numbers in organically rich sediments. It is interesting that relatively high aspartate aminotransferase activities were found in extracts of both species and it is speculated that maintenance of redox balance by means of the malate-aspartate shuttle (see DE ZWAAN, 1977) would be of advantage during periods of anaerobic metabolism wich may be necessary for survival as a consequence of the reduced oxygen supply when organic inputs are high.

The confident interpretation of the data and definition of biochemical characteristics of energetic advantage to each species in relation to their activity and fitness for survival in an organically rich environment requires further information on fluctuations in physiological concentrations of metabolites and their effects on key enzyme reactions. However, it is concluded that the maximal enzyme activities may have provided a useful preliminary indication of metabolic capability of each of the species in relation to muscular activity and the ability of *Capitella* and *Scolelepis* to survive periods when oxygen is in limited supply.

References

ALP, P.R., E.A NEWSHOLME and V.A. ZAMMIT, 1976. Activities of citrate synthase and NAD^+–linked and $NADP^+$–linked isocitrate dehydrogenase in muscle from vertebrates and invertebrates. Biochem. J. **154**, 689–700.

BLACKSTOCK, J., 1980. Estimation of activities of some enzymes associated with energy yielding metabolism in the polychaete *Glycera alba* (Müller) and application of the methods to the study of the effect of organic pollution. J. exp. mar. Biol. Ecol. **46**, 197–217.

PEARSON, T.H. and R. ROSENBERG, 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanogr. Mar. Biol. Ann. Rev. **16**, 229–311.

ZAMMIT, V.A. and E.A. NEWSHOLME, 1976. The maximum activities of hexokinase, phosphorylase, phosphofructokinase, glycerol phosphate dehydrogenases, lactate dehydrogenase, octopine dehydrogenase, phosphoenolpyruvate carboxykinase, nucleoside diphosphatekinase, glutamate-oxaloacetate transaminase and arginine kinase in relation to carbohydrate utilisation in muscles from marine invertebrates. Biochem. J. **160**, 447–462.

ZAMMIT, V.A., I. BEIS and E.A. NEWSHOLME, 1978. Maximum activities and effects of fructose biphosphate on pyruvate kinase from muscles of vertebrates and invertebrates in relation to the control of glycolysis. Biochem. J. **174**, 989–998.

ZWAAN, A. de, 1977. Anaerobic energy metabolism in bivalve molluscs. Oceanogr. Mar. Biol. Ann. Rev. **15**, 103–187.