flux of substrate through the phosphoenolpyruvate pathway, which is the main degradative route under this condition, will increase. The result for *M. edulis* differs from that obtained for *A. vinelandii* as far as the phosphoenolpyruvate → oxaloacetate step is concerned, and this difference fits very well with the role that this step plays in both organisms.

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Some Kinetic and Regulatory Properties of the Cytoplasmic L-Malate Dehydrogenases from the Posterior Adductor Muscle and Mantle Tissues of the Common Mussel *Mytilus edulis*

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The anaerobic metabolism of the common mussel *Mytilus edulis* is different from that of vertebrate skeletal muscle. Instead of causing an accumulation of lactate, phosphoenolpyruvate is converted into either pyruvate (and probably then into alanine) by pyruvate kinase or oxaloacetate by cytoplasmic phosphoenolpyruvate carboxykinase. Oxaloacetate is reduced to malate in the cytoplasm by L-malate dehydrogenase (EC 1.1.1.37), and malate (or fumarate) enters the mitochondrion and is reduced to succinate. Succinate is probably converted into propionate [for a review see de Zwaan *et al.* (1976)]. The phosphoenolpyruvate carboxykinase reaction is thought to predominate over the pyruvate kinase reaction with increasing anaerobiosis (de Zwaan & van Marrewijk, 1973; Livingstone & Bayne, 1974; Kluytmans *et al.*, 1975), and it is hypothesized that, by this metabolic scheme, the ATP yield per molecule of glucose 6-phosphate is increased (de Zwaan *et al.*, 1976; de Zwaan & Wijsman, 1976). The reduction of oxaloacetate to malate is a key reaction in the anaerobic pathway, and it is generally thought that in bivalves such as *Mytilus*, malate dehydrogenase replaces lactate dehydrogenase in reoxidizing cytoplasmic NADH (generated anoxically). The ratios of malate dehydrogenase/lactate dehydrogenase are always high in organisms forming succinate (see de Zwaan *et al.*, 1976). A study of malate dehydrogenase from two functionally different tissues of *Mytilus* was considered important for several reasons: (1) malate accumulation occurs in the posterior adductor muscle under certain conditions of hypoxia; (2) a more acute response of the posterior adductor muscle, compared with the mantle, to anaerobiosis has been indicated (D. R. Livingstone & B. L. Bayne, unpublished work); (3) it has been shown that bovine heart cytoplasmic malate dehydrogenase exists in an allosteric form which is inhibited by D-fructose 1,6-bisphosphate (Cassman, 1973; Cassman & Vetterlein, 1974; Vetterlein & Cassman, 1974).

Experimental

Homogenates of the two tissues were prepared in 0.88M-sucrose/0.01M-Tris/HCl, pH8.5 (4°C), containing 1mM-EDTA and 1mM-dithiothreitol. The mitochondria are unaffected structurally by this procedure (Addink & Veenhoff, 1975). After centrifugation (12000*g* and 40000*g* for 90min periods), the supernatants were partially purified by treatment with 1% protamine sulphate (1mg/60mg of soluble protein) and $(NH_4)_2SO_4$ fractionation (40–65%-satn.). These extracts were used for the kinetic studies after exhaustive dialysis against buffer. Crude supernatants of the tissues from individual mussels were also examined on a number of occasions. Lineweaver–Burk and Hill plots were fitted by least-squares analysis. The enzyme was assayed spectrophotometrically in the direction of malate formation by the decrease in absorption at 334nm due to NADH oxidation (Englard, 1969). The assay temperature was 25°C and Hepes* buffer was used at pH8.0, as Tris/HCl was inhibitory at low concentrations of oxaloacetate (Figs. 1*c* and 1*d*; Fig. 2, open circles).

Results and discussion

The optimal NADH concentration at pH8.0 was 0.2mM for both enzymes, and, above this, considerable substrate inhibition was observed. The optimal oxaloacetate concentration was 0.3–0.5mM for the posterior-adductor-muscle malate dehydrogenase and 0.5–1.0mM for the mantle malate dehydrogenase. Both enzymes displayed significant activity over a wide pH range (6.5–10) with the mantle profile displaced to alkaline pH relative to that of the posterior adductor muscle (Figs. 1*a* and 1*b*). The pH optima (8.0 and 9.0 for posterior adductor muscle and mantle respectively) showed no consistent differences from the malate dehydrogenases of other sources, being higher than those for the cytoplasmic enzymes of bovine kidney (pH7.4; Dupourque & Kun, 1969) and chicken

* Abbreviation: Hepes, 2-(*N*-2-hydroxyethylpiperazin-*N'*-yl)ethanesulphonic acid.

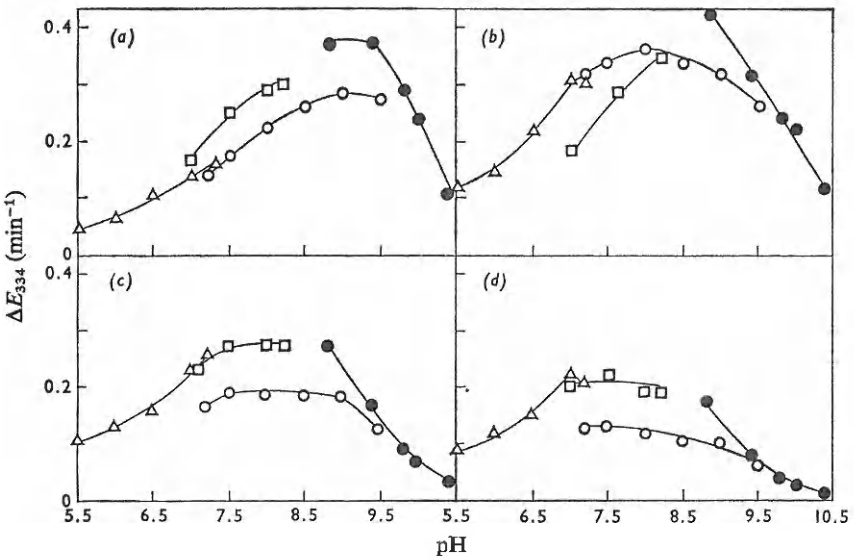

Fig. 1. *Changes in malate dehydrogenase activity with pH*

(*a*) and (*c*), mantle; (*b*) and (*d*), posterior adductor muscle. △, Sodium cacodylate/HCl; ○, Tris/HCl; ●, glycine/NaOH; □, Hepes. The incubation mixture contained 100mM buffers, 0.2mM-NADH and 1.0mM- (*a* and *b*) or 0.05mM- (*c* and *d*) oxaloacetate.

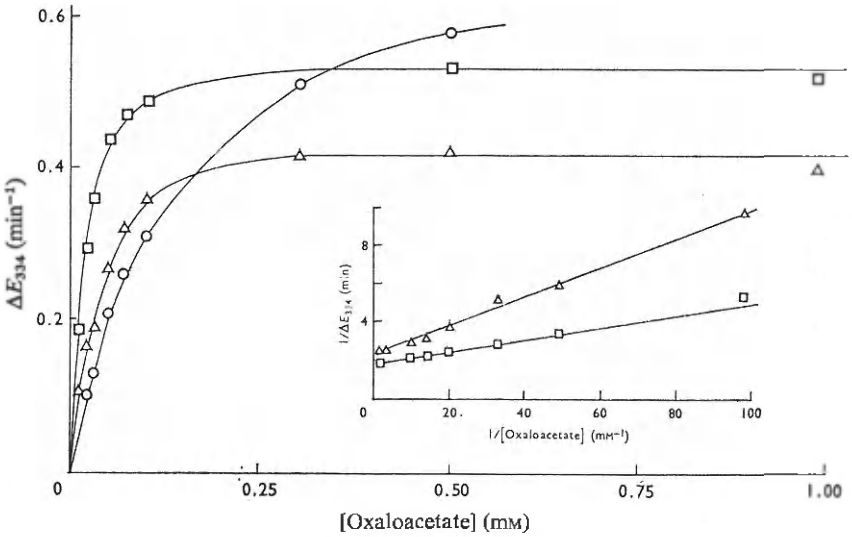

Fig. 2. *Changes in posterior-adductor-muscle malate dehydrogenase activity with oxaloacetate concentration at different pH values*

□, Hepes, pH8; ○, Tris/HCl, pH8; △, sodium cacodylate/HCl, pH6.5. Inset: Lineweaver–Burk plots.

heart (pH7.6; Kitto & Kaplan, 1966) and lower than those for *Bacillus subtilis* (pH9.6; Yoshida, 1965) and *Escherichia coli* (pH9.0; Murphey *et al.*, 1967). At more physiological oxaloacetate concentrations (0.05mM), the differences between the two enzymes were much less, and generally flatter pH profiles were obtained (Figs. 1*c* and 1*d*), ideally suited to the enzymes functioning in an environment of changing pH during anaerobiosis (see Wijsman, 1975).

The mantle malate dehydrogenase showed Michaelis–Menten kinetics at pH8.0 with respect to both oxaloacetate (Fig. 3 and inset, open squares) and NADH. In contrast, although the posterior-adductor-muscle malate dehydrogenase showed Michaelis–Menten kinetics with respect to oxaloacetate (Fig. 2 and inset, open squares), the situation for NADH appears complicated (results not shown). The correlation coefficients for the Lineweaver–Burk plots were consistently lower than for the mantle malate dehydrogenase, and for some preparations the results were better fitted to a Hill plot with Hill coefficients (h) of up to 1.5. On examination of the posterior-adductor-muscle malate dehydrogenase of ten individual mussels, a mean h of 1.3 ± 0.03 (S.E.) was determined, which was significantly higher ($0.1>P>0.05$) than the value for the mantle malate dehydrogenase data ($h=1.14\pm0.07$). Some preparations of the enzyme had curves of reaction velocity versus NADH with 'bumpy' shapes similar to those observed for the pyruvate kinase of the Alaskan king crab (Somero, 1969). This was interpreted by Somero (1969) as being due to the simultaneous presence of two enzyme variants, one displaying sigmoidal, and the other hyperbolic kinetics. The cytoplasmic malate dehydrogenase of bovine heart also exists in two forms, which display different kinetics with respect to NADH, i.e. malate dehydrogenase *a* (Michaelis–Menten) and malate dehydrogenase *b* (sigmoidal). The two forms can be present in a single preparation and are structurally identical except for their degree of phosphorylation (Cassman & Vetterlein, 1974; Vetterlein & Cassman, 1974). The situation for the posterior-adductor-muscle malate dehydrogenase is not yet resolved, and the apparent K_m values for NADH quoted below are derived from Lineweaver–Burk plots. However, the existence of an

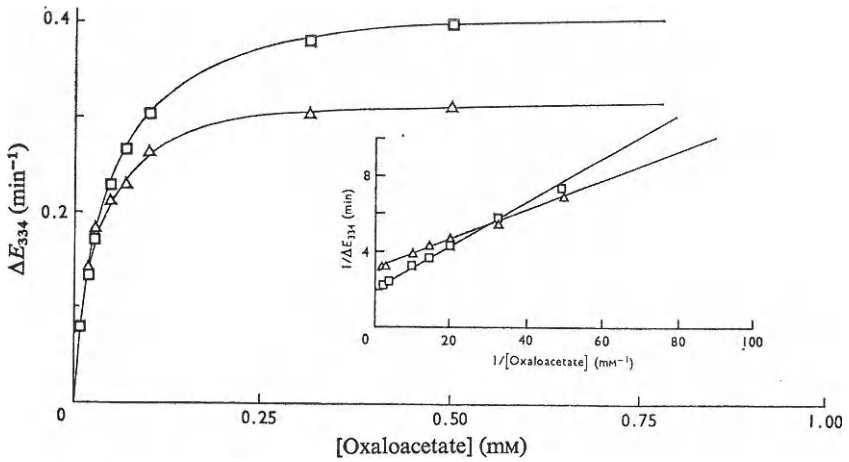

Fig. 3. *Changes in mantle malate dehydrogenase activity with oxaloacetate concentration at different pH values*

□, Hepes, pH 8; △, sodium cacodylate/HCl, pH 6.5. Inset: Lineweaver–Burk plots.

enzyme variant in this tissue, displaying sigmoidal kinetics with respect to NADH, would clearly be of physiological significance, as such an enzyme would be more sensitive to the large and sudden changes in NADH concentration that are likely to occur at the onset of anaerobiosis. It would also imply that in this respect the posterior-adductor-muscle malate dehydrogenase is better adapted to anaerobic metabolism than the mantle enzyme.

The apparent K_m values for NADH at pH 8.0 of the two isoenzymes were almost identical, i.e. $32.4 \pm 1.4\,\mu$M (posterior adductor muscle) and $31.3 \pm 1.8\,\mu$M (mantle). In contrast, the apparent K_m for oxaloacetate of the mantle enzyme was higher than that of the posterior-adductor-muscle enzyme and varied with the time of year that the animals were collected, i.e. $23.1 \pm 1.3\,\mu$m (posterior adductor muscle) and for the mantle, $46.8\,\mu$M (May) and $135.0 \pm 9.4\,\mu$M (November). The substrate affinities were generally similar to those of other malate dehydrogenases, e.g. bovine heart cytoplasmic (pH 9.0) (Grimm & Doherty, 1961): $51\,\mu$M (oxaloacetate) and $38\,\mu$M (NADH); *Bacillus subtilis* (pH 8.8) (Yoshida, 1965): $61\,\mu$M (oxaloacetate) and $27\,\mu$M (NADH). The lower affinity of the mantle enzyme for oxaloacetate would seem particularly significant in view of the biosynthetic nature of this tissue. Unlike the posterior adductor muscle, whose function is mainly one of valve closure, the mantle tissue is the site of gametogenesis, and large changes in carbohydrate, lipid, protein and nucleic acid concentrations occur with season (Thompson, 1972; Gabbott & Bayne, 1973). The lowered affinity for oxaloacetate would (1) allow increased use of this metabolite for transamination to aspartate, which is a necessary precursor for pyrimidine and purine biosynthesis, and (2) suggest that the malate dehydrogenase reaction is more reversible in this tissue. The possibility of function (1) is strongly supported by the striking increase in the apparent K_m for oxaloacetate in November ($135\,\mu$M) compared with the value for May ($46.8\,\mu$M). Nucleic acid synthesis is most active in the mantle in late autumn and least active in the spring after spawning (Thompson, 1972). The apparent k_m values for NADH were the same at both times of the year. The increased reversibility of the reaction would be important in relation to the function of cytoplasmic malate dehydrogenase in gluconeogenesis, i.e. the pathway involves carboxylation of pyruvate to oxaloacetate in the mitochondrion, transportation out as malate, and oxidation of malate back to oxaloacetate by malate dehydrogenase in the cytoplasm for conversion into phosphoenolpyruvate and glucose (Lardy *et al.*, 1965).

Malate can form oxaloacetate in the cytoplasm, despite an unfavourable equilibrium constant, because of the high ratios of malate/oxaloacetate and of NAD/NADH. In the direction of oxaloacetate formation, it is noteworthy that, although the posterior-adductor-muscle malate dehydrogenase is inhibited by high concentrations of malate (>2mM), the mantle enzyme is not (Livingstone, 1975). An homologous situation is observed for the chicken heart cytoplasmic and mitochondrial malate dehydrogenases respectively (Kitto & Kaplan, 1966). In general agreement with the differences in apparent K_m for oxaloacetate, the oxaloacetate concentrations tend to be higher in the mantle tissue than the adductor muscle, i.e. 0.017–0.032 μmol/g (mantle; Bayne, 1973) and 0.0029–0.028 μmol/g (posterior adductor muscle; Livingstone, 1975).

Differences were also observed with respect to the change in apparent K_m values for oxaloacetate with a decrease in pH. Whereas the apparent K_m for oxaloacetate of the posterior-adductor-muscle malate dehydrogenase increased slightly (22.0 μM to 36.7 μM at pH6.5; Fig. 2), that of the mantle malate dehydrogenase decreased to a low value (46.8 μM to 26.8 μM at pH6.5, Fig. 3; also 135.0 to 76.8 for the November sample). These differences are clearly illustrated in the curves of velocity versus substrate at the two pH values and in the Lineweaver–Burk plots (Figs. 2 and 3 and insets). The value for the mantle malate dehydrogenase at pH6.5 was similar to that for the posterior-adductor-muscle malate dehydrogenase at pH8.0. At pH6.5 the optimal oxaloacetate concentration for the mantle enzyme (determined at pH8.0) was in fact inhibitory (Figs. 1*a* and 1*c*, pH6.5 values). The apparent K_m values for NADH of both enzymes changed little with pH. With decreasing pH the mantle enzyme therefore becomes better suited to operating in the direction of oxaloacetate reduction, as is required for anaerobic metabolism.

The results of the enzyme studies are consistent with the different functions of the tissues and their response to anaerobiosis. Although the posterior-adductor-muscle enzyme appears to operate predominantly in the direction of malate formation at all pH values, the mantle enzyme has the apparent capacity to revert to biosynthetic functions with a return to aerobic conditions. The possibility of sigmoidicity with respect to NADH and the inhibitory effect of aspartate (results not shown) on the posterior-adductor-muscle malate dehydrogenase also suggest that the muscle tissue will respond more immediately to changes in oxygen availability.

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MICROBIAL CATABOLISM: ITS ROLE IN THE CARBON CYCLE AND ENVIRONMENTAL SIGNIFICANCE: a Colloquium organized by P. A. Williams (Bangor)

Introduction: Anaerobic and Aerobic Environments

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Life on Earth depends on a dynamic balance between living organisms possessing a diversity of biochemical competence, and the environment. This 'throughput' of chemicals from inanimate materials (rocks, soils, natural waters and air, with the aid of solar radiation) to their participation in biochemical processes and their subsequent mineralization again, has evolved over a geological time-scale and under a variety of physical conditions.

The constituents of living organisms must be susceptible to degradation or they would eventually accumulate on the surface of the earth and thus lock the chemicals in an unavailable form. Micro-organisms are the scavengers *par excellence* in maintaining the carbon cycle, producing eventually CO_2 and methane from all naturally occurring organic compounds.

Man is introducing increasingly large amounts of synthetic organic chemicals into the environment, particularly as herbicides, pesticides, detergents and the unwanted by-products of industry. Some of these molecules are recalcitrant to microbial attack and therefore accumulate in the biosphere; their effects (apart from any direct toxicity to man) on normal food-chains are not easy to predict, but have been disastrous in some instances.

Biodegradability is clearly obligatory for soluble, potentially toxic, organic chemicals if they are to be used by man for whatever purpose, and are likely to be disseminated in the environment.

Microbial catabolism is a wide topic; it seems appropriate to focus attention on some of the fundamental biochemical aspects of the central 'carbon' cycle. Guiding principles may emerge that have a bearing on biodegradability in Nature.

Carbon cycle

Scheme 1 illustrates the biological carbon cycle. The principal geochemical reservoirs of carbon have been quoted as follows (Doetsch & Cook, 1973):

Atmosphere $= 2.3 \times 10^{18}$ g (as CO_2)

Biosphere $= 2.6 \times 10^{17}$ g (inorganic and organic C)

Lithosphere $= 1.2 \times 10^{22}$ g (sedimentary rocks and carbonates)

$= 3.6 \times 10^{21}$ g (fossil fuels: coal, petroleum and kerogen)

$= 1.3 \times 10^{22}$ g (igneous and metamorphic rocks)

The smallest segment with respect to the amount of this element is the biosphere. Carbon enters the biosphere primarily by photosynthesis (terrestrial plants, aquatic algae and especially the unicellular marine diatoms and dinoflagellates), i.e. by the reduction of CO_2; the contribution of photosynthetic and chemolithotrophic bacteria is thought to be relatively small in comparison. Estimates of CO_2 fixation vary widely; possibly 5×10^{16}–15×10^{16} g of carbon enters the biosphere annually by photosynthesis