# Division of Comparative Physiology and Biochemistry, Society for Integrative and Comparative Biology

Organ-Specific Analysis of the Time Course of Covalent Modification of Pyruvate Kinase during Anaerobiosis in a Marine Whelk

Author(s): Ross E. Whitwam and Kenneth B. Storey

Source: *Physiological Zoology*, Vol. 63, No. 1 (Jan. - Feb., 1990), pp. 222-234
Published by: The University of Chicago Press . Sponsored by the Division of Comparative Physiology and Biochemistry, Society for Integrative and Comparative Biology
Stable URL: http://www.jstor.org/stable/30158163
Accessed: 22-03-2016 16:03 UTC

# Organ-specific Analysis of the Time Course of Covalent Modification of Pyruvate Kinase during Anaerobiosis in a Marine Whelk

**Ross E. Whitwam**
**Kenneth B. Storey**

Institute of Biochemistry and Department of Biology, Carleton University, Ottawa, Ontario, Canada K1S 5B6

Accepted 6/13/89

**Abstract**

*The progress of anoxia-induced phosphorylation of pyruvate kinase (PK) was monitored in four organs of the whelk* Busycotypus canaliculatum *over the course of 21 h of exposure to $N_2$-bubbled seawater. In all cases, PK modification resulted in a decrease in affinity for phosphoenolpyruvate (PEP) (the substrate affinity constant, $S_{0.5}$, rose by 3–6-fold) and a strong increase in inhibition by alanine (the inhibitor constant, $I_{50}$, decreased to 0.1%–13% of the aerobic value). However, the time course of kinetic changes was distinctly different for each of the three isozymic forms tested (ventricle, foot, and hepatopancreas/kidney), and, in all cases, anoxia-induced modification of the $I_{50}$(Ala) occurred much more rapidly than did changes in $S_{0.5}$(PEP). Enzyme modification occurred most rapidly in hepatopancreas and kidney with half-times for the decrease in $I_{50}$(Ala) of 20–25 min and for the increase in $S_{0.5}$(PEP) of 100 min. Comparable values for ventricle were 110 and 210 min and for foot were 225 and 550 min for $I_{50}$(Ala) and $S_{0.5}$(PEP), respectively. With two phosphorylation sites per subunit, these data suggest that anoxia-induced enzyme phosphorylation occurs first at a site that influences $I_{50}$(Ala). The initial response to anoxia exposure by PK in both ventricle and foot included a change in kinetic properties that was opposite to the final result after long-term anoxia. In ventricle, for example, $S_{0.5}$(PEP) decreased and $I_{50}$(Ala) increased within the first 30 min; by 2 h, however, these changes had reversed and kinetic parameters were progressing toward the anoxic end points. These data are consistent with a biphasic response by metabolism over the aerobic-anaerobic transition in ventricle and foot: an initial activation of PK (and of glycolytic rate) as conditions become increasingly hypoxic, followed by PK inactivation (and glycolytic rate depression) to permit long-term anaerobiosis.*

*Physiological Zoology* 63(1):222–234. 1990.  0031-935X/90/6301-8915$02.00

## Introduction

Pyruvate kinase (PK) has a central role in the control of fermentative metabolism in anoxia-tolerant marine molluscs. Regulation at PK controls the partitioning of carbon flow at the phosphoenolpyruvate (PEP) branch point into pyruvate-derived (L-alanine) versus oxaloacetate-derived (succinate, propionate) end products (De Zwaan 1983; De Zwaan and Dando 1984). Anoxia-induced inactivation of PK is also a key part of the glycolytic rate depression (the Pasteur effect does not occur) and overall metabolic rate depression that characterizes facultative anaerobiosis (Storey 1985). The regulatory mechanisms involved in the control of PK have been examined in a number of species of anoxia-tolerant marine molluscs, with the most detailed attention given to the bivalve *Mytilus edulis* and the gastropod *Busycotypus canaliculatum.* Initial studies focused on the effects of pH and allosteric inhibitors (L-alanine) in enzyme control; both the drop in cellular pH and rapid increase in levels of the fermentative product, L-alanine, inhibit PK activity in the anoxic animal (Hochachka and Mustafa 1972; De Zwaan and Dando 1984). Subsequent studies showed the overriding importance of anoxia-induced enzyme phosphorylation in converting the enzyme into a much less active form (Holwerda et al. 1983; Plaxton and Storey 1984*a,* 1984*b,* 1985*a,* 1985*b;* Hakim et al. 1985). In the whelk *B. canaliculatum,* anoxia-induced modification of PK was indicated in all organs tested and included all three tissue-specific isozymic forms of PK (Plaxton and Storey 1985*a*). For example, compared to the aerobic, dephosphorylated enzyme, the anoxic, phosphorylated, variant of PK from radular retractor muscle showed a threefold lower maximal activity, reduced affinity for PEP ($S_{0.5}$ 12-fold higher), reduced activation by fructose-1,6-bisphosphate ($K_a$ 26-fold higher), and extremely potent inhibition by L-alanine ($K_i$ 490-fold lower) (Plaxton and Storey 1984*a*).

Although the phenomenon of anoxia-induced phosphorylation of PK has now been well demonstrated, the progress of the conversion from the aerobic to the anoxic enzyme form has not been characterized. The present study follows the time course of changes in the kinetic parameters of PK from four organs of the whelk over 21 h of environmental anoxia. The results show both organ-specific differences in the progress of PK modification during anoxia and distinct differences in the rate of change of two kinetic parameters, the $S_{0.5}$(PEP) versus the $I_{50}$(Ala). Both can have important consequences for the regulation of carbon flow during the aerobic-anaerobic transition.

## Material and Methods

*Chemicals and Animals*

Chemicals were obtained from J.T. Baker Chemical Co., Boehringer Mannheim GmbH, or Sigma Chemical Co. Channeled whelks, *Busycotypus canaliculatum,* were obtained from the Marine Biological Laboratory, Woods Hole, Massachusetts, and were kept until use in recirculating aerated seawater (1,000 mosm) at 12°–15°C without feeding. Aerobic whelks were taken directly from the seawater tank. Tissues were dissected out and immediately frozen in liquid nitrogen; complete dissection took 3–5 min. Tissues were stored until use at −80°C. For anoxia experiments whelks were placed in a large sealed tub filled with seawater that had been deoxygenated by bubbling with a steady stream of $N_2$ gas for the previous 12–16 h. The flow of nitrogen gas was maintained throughout the experimental time course. After a timed period of anoxia, the whelks were removed from the tub and killed as above.

Frozen tissues were ground to a powder under liquid nitrogen and homogenized (1:5 w/v for ventricle and foot, 1:2 w/v for hepatopancreas and kidney) in 50 mM imidazole-HCl buffer (pH 7.0) containing 100 mM NaF, 5 mM EDTA, 5 mM EGTA, 15 mM 2-mercaptoethanol, and 0.1 mM phenylmethylsulfonyl fluoride. Ammonium sulfate was added to hepatopancreas and kidney homogenates to 20% saturation, and the homogenates were stirred at 5°C for 30 min and then centrifuged; this treatment removed nonspecific background activity from the preparation but did not alter total PK activity. Foot and ventricle preparations did not require such treatment.

All homogenates were centrifuged at 27,000 *g* for 30 min at 5°C. Supernatants were removed and dialyzed overnight against 1,000 volumes of 50 mM imidazole-HCl buffer (pH 7.0) containing 50 mM NaF, 5 mM EDTA, 5 mM EGTA, and 15 mM 2-mercaptoethanol. Following dialysis, extracts of hepatopancreas and kidney were again centrifuged as above before use. Overnight dialysis produced no significant change in the measureable PK activity of any preparation.

The PK activity was measured by coupling the reaction to lactate dehydrogenase (LDH) and following the decrease in O.D. (optical density) at 340 nm due to the oxidation of NADH. The standard assay mixture contained 50 mM imidazole-HCl buffer (pH 7.0), 50 mM KCl, 5 mM $MgCl_2$, 2 mM ADP, 0.2 mM NADH, and two units/mL LDH with varying amounts of PEP and/or L-alanine. The $S_{0.5}$ values for PEP were determined using Hill plots. The $I_{50}$ values for L-alanine were determined by the method of Job et al. (1978). Data are means ± SEM, for determinations on $n = 3$ individual animals at

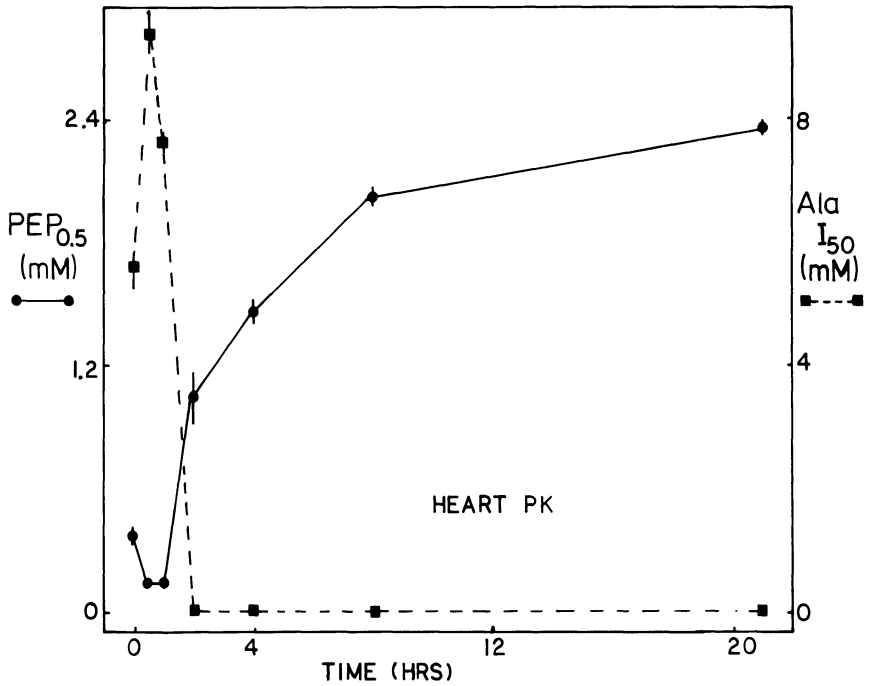


*Fig. 1. Time course of changes in the kinetic parameters of pyruvate kinase over 21 h of anoxia exposure in whelk ventricle. Data are means ± SEM for determinations on tissue from n = 3 individual animals; where error bars are not shown these are enclosed within the symbols used. Symbols are: ● —— ●, $S_{0.5}$ for PEP (mM); ■ —— ■, $I_{50}$ for L-alanine at 1.0 mM PEP (mM).*

each time point. Statistical significance was determined by the Student's *t*-test. The half-time ($t_{1/2}$) for change in a kinetic parameter was measured as the amount of time elapsed before an $I_{50}$ or $S_{0.5}$ value had reached the mid-way point between its initial and final values.

## Results

The changes in the substrate affinity constant for PEP, $S_{0.5}$(PEP), and the inhibitor constant for L-alanine, $I_{50}$(Ala), over a time course of anoxia are shown in figures 1–4 for PK from whelk ventricle, foot muscle, kidney, and hepatopancreas, respectively. In all four cases the response to prolonged anoxia was a large increase in the $S_{0.5}$(PEP) and a dramatic decrease in the $I_{50}$(Ala). Final values for these parameters after 21 h of anoxia (16 h for hepatopancreas) are shown in table 1 along with the aerobic control values. Fig-

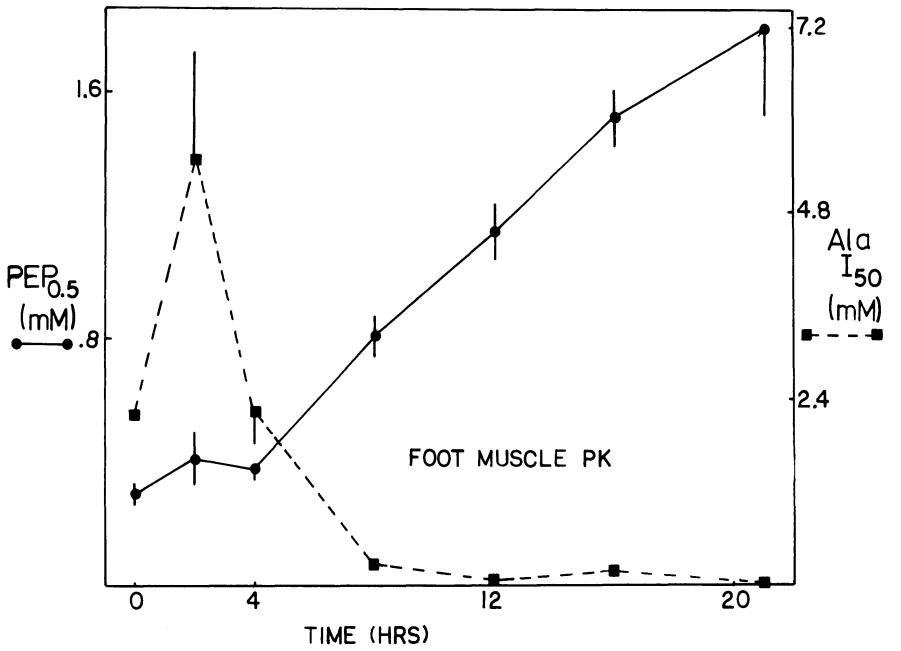


*Fig. 2. Time course of changes in the kinetic parameters of pyruvate kinase over 21 h of anoxia exposure in whelk foot muscle. For details see fig. 1. Symbols are: ● —— ●, $S_{0.5}$ for PEP (mM); ■ —— ■, $I_{50}$ for L-alanine at 2.0 mM PEP (mM).*

ures 1–4 show that the transition from aerobic to anoxic kinetic parameters was a gradual process; intermediate values for both kinetic parameters were seen and several hours were required before stable anoxic values were reached. Apparent half-times for the changes in $S_{0.5}$(PEP) and $I_{50}$(Ala) are shown in table 2. Two observations can be made. First, there are organ-specific differences in the $t_{1/2}$ values for both parameters; from the onset of anoxia, changes occur fastest in hepatopancreas and kidney, slower in ventricle, and slowest in foot muscle. Second, for all organs, the decrease in $I_{50}$(Ala) occurred much more rapidly than did the increase in $S_{0.5}$(PEP) values. The $t_{1/2}$ for the change in $S_{0.5}$(PEP) was twice as great as the $t_{1/2}$ for $I_{50}$(Ala) in ventricle and foot, and four times greater than the $t_{1/2}$ for $I_{50}$(Ala) in kidney and hepatopancreas.

Figures 1 and 2 also show that the initial responses to anoxia exposure by PK in ventricle and foot were in the opposite direction to those of the final anoxic result. Over the first 30 min of anoxia exposure, the $S_{0.5}$(PEP) of ventricle PK decreased by 50%, whereas the $I_{50}$(Ala) increased by 1.6-fold. By 2 h, however, the situation had reversed; the $I_{50}$(Ala) was depressed to its

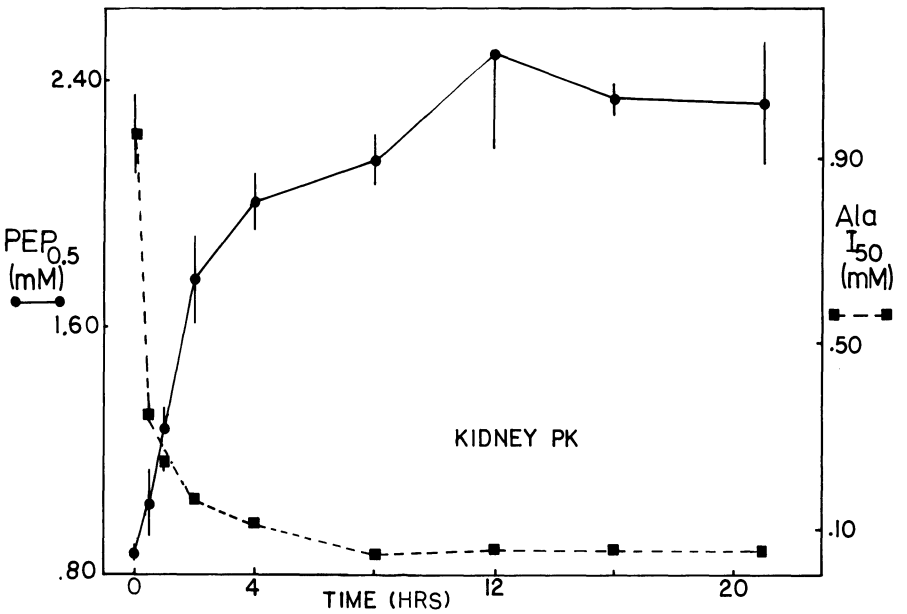


*Fig. 3. Time course of changes in the kinetic parameters of pyruvate kinase over 21 h of anoxia exposure in whelk kidney. For details see fig. 1. Symbols are:* ● —— ●, *$S_{0.5}$ for PEP (mM);* ■ —— ■, *$I_{50}$ for L-alanine at 2.0 mM PEP (mM).*

final anoxic value and the $S_{0.5}$(PEP) had increased to about 1.1 mM. At the end of 21 h of anoxia, the $I_{50}$(Ala) was reduced to 0.006 mM, fully three orders of magnitude below the control, aerobic value (table 1). Foot muscle PK showed the same pattern with respect to $I_{50}$(Ala). The $I_{50}$(Ala) increased twofold over the first 2 h of anoxia and then subsequently fell to a stable low value by 12 h. The $S_{0.5}$(PEP) responded differently and was unchanged over the first 4 h of anoxia exposure with the first significant increase in $S_{0.5}$(PEP) seen after 8 h of anoxia.

The curves detailing the kinetic changes in hepatopancreas PK and in kidney PK were virtually identical. Neither showed any initial changes in $S_{0.5}$(PEP) or $I_{50}$(Ala) that were opposite to the net effect of anoxia. Both the pattern of kinetic changes over the anoxic time course and the $t_{1/2}$ values for the changes in $S_{0.5}$(PEP) and $I_{50}$(Ala) were virtually identical. Polyacrylamide-gel electrophoresis and isoelectrofocusing studies have previously indicated that hepatopancreas and kidney share the same isozymic form of the enzyme (Plaxton and Storey 1985*a*), and the present data are consistent with this conclusion. However, differences in the absolute values for the

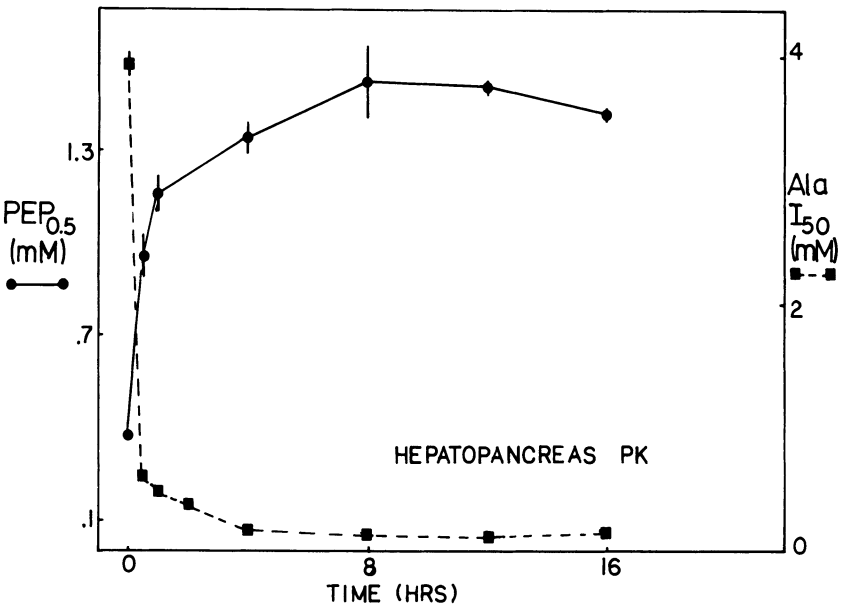


*Fig. 4. Time course of changes in the kinetic parameters of pyruvate kinase over 16 h of anoxia exposure in whelk hepatopancreas. For details see fig. 1. Symbols are: ● —— ●, $S_{0.5}$ for PEP (mM); ■ —— ■, $I_{50}$ for L-alanine at 2.0 mM PEP (mM).*

kinetic constants of the two forms suggest a somewhat higher "resting" content of phosphate bound to the kidney enzyme in the aerobic animal.

## Discussion

Exposure to anoxia alters the phosphorylation state of at least four enzymes in the whelk: PK, glycogen phosphorylase, 6-phosphofructo-1-kinase (PFK-1), and 6-phosphofructo-2-kinase (PFK-2) (Plaxton and Storey 1984*a*, 1984*b*, 1985*a*, 1985*b*; Storey 1984, 1988; L. Bosca and K. B. Storey, unpublished data). In all cases, the net effect, when anoxia is prolonged, is the conversion of the enzymes to less active forms in vivo. Action at these four loci is critical to the depression of glycolytic rate and, along with other regulatory mechanisms (e.g., changes in the association of enzymes with subcellular particles, depression of fructose-2,6-bisphosphate content), is responsible for the absence of a Pasteur effect in marine invertebrate facultative anaerobes (Storey 1985). Indeed, recent studies have shown that inactivation of these enzymes is coordinated during the aerobic-anaerobic transition

TABLE 1
*Effect of anoxia on the kinetic parameters of pyruvate kinase from four organs of the whelk*

| | $S_{0.5}$ (PEP) (mM) | | $I_{50}$ (Ala) (mM) | |
|---|---|---|---|---|
| | Aerobic | Anoxic | Aerobic | Anoxic |
| Ventricle | .38 ± .04 | 2.35 ± .03 | 5.51 ± .37 | .006 ± .0002 |
| Foot muscle | .30 ± .04 | 1.80 ± .28 | 2.21 ± .08 | .28 ± .006 |
| Kidney | .87 ± .03 | 2.33 ± .20 | .95 ± .09 | .053 ± .003 |
| Hepatopancreas | .38 ± .01 | 1.41 ± .02 | 3.90 ± .20 | .12 ± .007 |

Note. Values are means ± SEM; $n = 3$. Anoxia exposure was 16 h for hepatopancreas and 21 h for other organs. Assay conditions are as in figs. 1–4.

in each organ; thus, the fall in $V_{max}$ activity of PFK-2 and PK followed parallel courses over 21 h of anoxia in whelk gill (L. Bosca and K. B. Storey, unpublished data). The regulatory mechanism involved is reversible protein phosphorylation, affecting not only these four enzymes but a variety of other cellular proteins to contribute to the overall metabolic rate depression that underlies long-term anaerobiosis (Plaxton and Storey 1984*b*). Phosphorylation inactivation of PK, PFK-1, and PFK-2 is mediated by the actions of an

TABLE 2
*Apparent half-times for the changes in kinetic parameters of pyruvate kinase during anoxia in four organs of the whelk*

| | Half-time (min) | |
|---|---|---|
| Organ | $S_{0.5}$ (PEP) | $I_{50}$ (Ala) |
| Ventricle | 210 | 110 |
| Foot | 550 | 225 |
| Kidney | 100 | 25 |
| Hepatopancreas | 100 | 20 |

endogenous protein kinase, but this is not the cyclic AMP-dependent protein kinase (L. Bosca and K. B. Storey, unpublished data; Brooks and Storey 1989).

Anoxia-induced modification of PK occurs in all organs of the whelk and affects all three isozymic forms: the red-muscle (occurring in ventricle, radular retractor muscle), white-muscle (foot, mantle), and soft-tissue (gill, hepatopancreas, kidney) enzymes (Plaxton and Storey 1985*a*). Studies with $^{32}P$ incorporation have proved the phosphoprotein nature of the anoxic form of PK in radular retractor muscle (Plaxton and Storey 1984*b*); treatments with agents that stimulate protein kinase activity similarly support the dephosphorylated versus phosphorylated nature of the aerobic versus anoxic enzyme in other organs (Brooks and Storey 1989).

Detailed studies of the radular retractor and hepatopancreas enzymes have shown that covalent modification affects $V_{max}$ and $S_{0.5}$(PEP) as well as the kinetic constants for a variety of activators and inhibitors of the enzyme (Plaxton and Storey 1984*a,* 1985*b*). After 21 h of anoxia, 7.3 moles of phosphate were incorporated per mole of tetrameric radula retractor muscle PK, or approximately 2 mol/subunit (Plaxton and Storey 1984*b*). The present study shows that the change in kinetic properties of the enzyme is gradual, requiring several hours and with intermediate states occurring. The same result was implied in studies of *Mytilus edulis* and *M. galloprovincialis;* the maximal activity of PK in adductor muscle dropped over the course of several hours of anoxia (Holwerda, Veenhof, and De Zwaan 1984; De Vooys and Holwerda 1986). This suggests that enzyme properties are modified in a stepwise fashion as phosphorylation is extended to each of the protomers of the enzyme tetramer, possibly including an influence of phosphorylated protomers on the conformation and kinetic properties of unphosphorylated subunits. The present data also show distinct differences in the time coefficients for the changes in $S_{0.5}$(PEP) versus $I_{50}$(Ala). The increase in $S_{0.5}$(PEP) was always slower than the change in $I_{50}$(Ala) for all organs; $t_{1/2}$ values for $S_{0.5}$(PEP) were 2-fold greater in ventricle and foot and 4–5-fold greater in kidney and hepatopancreas than the corresponding $t_{1/2}$ for $I_{50}$(Ala). Since there are apparently two phosphorylation sites per subunit of whelk PK (Plaxton and Storey 1984*b*), the molecular basis of this result may be differential rates of anoxia-induced phosphorylation at the two sites. The site that influences the binding of the allosteric inhibitor, L-alanine, may be rapidly phosphorylated, whereas phosphorylation at a second site that influences PEP binding at the active site appears to be a slower event.

Some note of how this compares with mammalian PK should be made. Liver-type PK, but not the muscle type, is subject to control by both allosteric effectors and reversible phosphorylation. Phosphorylation of liver PK results in the incorporation on 1 mol of phosphate per mole of PK subunit and alters kinetic parameters, increasing $S_{0.5}$(PEP) about threefold, lowering $I_{50}$(Ala) by 50%, and raising $K_a$ fructose-1,6-$P_2$ by twofold (Engstrom et al. 1987). Obviously, the effect of anoxia-induced phosphorylation on the kinetic constants for inhibitors and activators of PK in the whelk is much more dramatic than that seen for the mammalian enzyme. It appears that this comes from the addition of a second phosphorylation site on each subunit, one that appears to confer, in particular, very dramatic changes in enzyme sensitivity to alanine. This, of course, would be particularly useful in regulating flux at the PEP branch point in anoxia-tolerant marine molluscs. Both the accumulation of alanine as the primary end product of the early hours of anoxia (Eberlee and Storey 1988; Storey et al. 1989) and the anoxia-induced phosphorylation of PK to create a strongly alanine-sensitive enzyme should combine to effectively shut down flux through PK and promote instead carbohydrate fermentation to succinate or propionate as end products. However, with such strong inhibition of the phosphoenzyme by alanine, how, in fact, does alanine accumulate as an end product in anoxia? Indeed, the time course of alanine accumulation in whelk ventricle and foot (Eberlee and Storey 1988) runs nearly parallel to the pattern of change seen for the $S_{0.5}$(PEP) and not to the much more rapid change in $I_{50}$(Ala). However, in ventricle, about 75% of the total alanine accumulation occurred within the first 4 h of anoxia exposure, and perhaps before the enzyme becomes extremely sensitive to alanine inhibition. An alternative route of alanine formation in anoxia has been proposed by De Zwaan (1983). This is via the decarboxylation of malate in the mitochondria. In this scheme an early phosphorylation inactivation of PK would function to shunt all glycolytic carbon via phosphoenolpyruvate carboxykinase into the formation oxaloacetate. Transported into the mitochondria as malate the resulting division of carbon flow into alanine versus succinate products would probably depend on the availability of amino groups (derived from aspartate) to support alanine production, with alanine accumulation slowing in a mirror image of aspartate depletion (the observed pattern in whelk organs) (Eberlee and Storey 1988).

The initial response to anoxia exposure by PK in ventricle and foot was opposite to the final result after 21 h of anaerobiosis. The $I_{50}$(Ala) in both organs increased significantly, and $S_{0.5}$(PEP) fell in ventricle. The physical

basis of this effect is probably an initial dephosphorylation of the enzyme, removing what little phosphate remained covalently bound to the aerobic enzyme form. Indeed, Plaxton and Storey (1984*b*) have previously shown that the aerobic form of PK is, in fact, partially phosphorylated, the enzyme from radular retractor muscle incorporating about 10%–15% of the $^{32}P$ label found in the anoxic enzyme form. The physiological result of these changes in enzyme kinetic parameters would be an initial activation of PK occurring with the first exposure to anoxic water. Indeed, data from metabolite studies show that the first response to immersion in $N_2$-bubbled seawater is, in fact, an activation of glycolysis, occurring during the hypoxic transition period. Changes in substrate and product levels for both PFK-1 and PK in foot and radular retractor muscle indicated an increased flux through both loci within 30–60 min (Storey et al. 1989). This is actually the oxygen-conforming strategy used by many animals in which the rates of glycolysis and of oxidative phosphorylation vary in an inverse manner over a wide range of low oxygen tensions in order to maintain constant ATP output (and constant metabolic rate) (Hochachka 1988). When oxygen tension drops below a critical limit, however, facultative anaerobes adopt a new strategy and conserve energy by metabolic rate depression (Shick, De Zwaan, and De Bont 1983; Storey 1985). Thus, after 2–4 h of exposure to anoxic water metabolic changes in whelk organs indicate a glycolytic rate depression (Storey et al. 1989), despite the need to rely totally on fermentative pathways for ATP generation. In the fully anoxic situation, then, glycolytic rate is actually lower than the rate in the aerobic organ, and the Pasteur effect (an activation of glycolysis in response to anoxia) does not occur. The time elapsed before metabolic depression responses are initiated varies between organs, being less than 1 h for hepatopancreas and kidney, between 1 and 2 h in ventricle, and greater than 4 h in foot. The reasons for these differences may include organ differences in aerobic metabolic rate as well as organ differences in the threshold oxygen tension that stimulates metabolic depression.

## Acknowledgments

Thanks to J. M. Storey for critical reading of the manuscript. Supported by an operating grant from the NSERC Canada to K.B.S.

## Literature Cited


BROOKS, S. P. J., and K. B. STOREY. 1989. Influence of hormones, second messengers, and pH on the expression of metabolic responses to anoxia in a marine whelk. J. Exp. Biol. (in press).

DE VOOYS, C. G. N., and D. A. HOLWERDA. 1986. Anaerobic metabolism in sublittoral living *Mytilus galloprovincialis* lam. in the Mediterranean. III. The effect of anoxia and osmotic stress on some kinetic parameters of adductor muscle pyruvate kinase. Comp. Biochem. Physiol. 85B:217–221.

DE ZWAAN, A. 1983. Carbohydrate metabolism in bivalves. Pages 137–175 in K. M. WILBUR, ed. The Mollusca. Vol. 1. Academic Press, New York.

DE ZWAAN, A., and P. R. DANDO. 1984. Phosphoenolpyruvate-pyruvate metabolism in bivalve molluscs. Mol. Physiol. 3:285–310.

EBERLEE, J. C., and K. B. STOREY. 1988. Tissue-specific biochemical responses during anoxia and recovery in the channeled whelk. J. Exp. Mar. Biol. Ecol. 121:165–176.

ENGSTROM, L., P. EKMAN, E. HUMBLE, and O. ZETTERQVIST. 1987. Pyruvate kinase. Pages 47–75 in P. D. BOYER and E. G. KREBS, eds. The enzymes. Academic Press, New York.

HAKIM, G., E. CARPENE, P. CORTES, and G. HSANI. 1985. Regulation by phosphorylation-dephosphorylation of pyruvate kinase in *Venus gallina* and *Scapharca inaequivalvis*. Comp. Biochem. Physiol. 80B:109–112.

HOCHACHKA, P. W. 1988. Metabolic suppression and oxygen availability. Can. J. Zool. 66:152–158.

HOCHACHKA, P. W., and T. MUSTAFA. 1972. Invertebrate facultative anaerobiosis. Science 178:1056–1060.

HOLWERDA, D. A., P. R. VEENHOF, and A. DE ZWAAN. 1984. Physiological and biochemical investigations of the ecological relevance of anaerobiosis in bivalves. I. The changes in activity of mussel adductor muscle and mantle pyruvate kinase during aerial exposure and reimmersion. Mar. Biol. Lett. 5:185–190.

HOLWERDA, D. A., P. R. VEENHOF, H. A. A. VAN HEUGTEN, and D. I. ZANDEE. 1983. Modification of mussel pyruvate kinase during anaerobiosis and after temperature acclimation. Mol. Physiol. 3:225–234.

JOB, D., C. CROCHET, A. DHIEN, and E. M. CHAMBAZ. 1978. A rapid method for screening inhibitor effects: determination of $I_{50}$ and its standard deviation. Anal. Biochem. 84:68–77.

PLAXTON, W. C., and K. B. STOREY. 1984*a*. Purification and properties of aerobic and anoxic forms of pyruvate kinase from red muscle tissue of the channelled whelk, *Busycotypus canaliculatum*. Eur. J. Biochem. 143:257–265.

———. 1984*b*. Phosphorylation in vivo of red-muscle pyruvate kinase from the channelled whelk, *Busycotypus canaliculatum*, in response to anoxic stress. Eur. J. Biochem. 143:267–272.

———. 1985*a*. Tissue-specific isozymes of pyruvate kinase in the channelled whelk, *Busycotypus canaliculatum:* enzyme modification in response to anoxia. J. Comp. Physiol. 155B:291–296.

———. 1985*b*. Purification and properties of aerobic and anoxic forms of pyruvate

kinase from the hepatopancreas of a channelled whelk, *Busycotypus canaliculatum.* Arch. Biochem. Biophys. 243:195–205.

Shick, J. M., A. De Zwaan, and A. M. T. De Bont. 1983. Anoxic metabolic rate in the mussel *Mytilus edulis* L. estimated by simultaneous direct calorimetry and biochemical analysis. Physiol. Zool. 56:56–63.

Storey, K. B. 1984. Phosphofructokinase from foot muscle of the whelk, *Busycotypus canaliculatum:* evidence for covalent modification of the enzyme during anaerobiosis. Arch. Biochem. Biophys. 235:665–672.

———. 1985. A re-evaluation of the Pasteur effect: new mechanisms in anaerobic metabolism. Mol. Physiol. 8:439–461.

———. 1988. Mechanisms of glycolytic control during facultative anaerobiosis in a marine mollusc: tissue specific analysis of glycogen phosphorylase and fructose-2,6-bisphosphate. Can. J. Zool. 66:1767–1771.

Storey, K. B., D. A. Kelly, J. A. Duncan, and J. M. Storey. 1989. Anaerobiosis and organ-specific regulation of glycolysis in a marine whelk. Can. J. Zool. (in press).