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# Ketoglutarate Dehydrogenase From Ribbed Mussel Gill Mitochondria: Modulation by Adenine Nucleotides and Calcium Ions

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*ABSTRACT* The transient accumulation of proline in the gills of the osmotically stressed ribbed mussel (*Modiolus demissus*), may be controlled by a transient regulation of the $\alpha$-ketoglutarate dehydrogenase (KGDH) activity. In this study, KGDH was partially purified from lysed mitochondria of gill tissue by differential centrifugation techniques. Various modulators of enzyme activity assayed at a saturating concentration (2.5 mM) of ketoglutarate (KG), showed inhibition by high concentrations of Cl (10–100 mM) and a slight activation or inhibition by the other compounds (10 mM), when assayed in the absence of added $Ca^{++}$. Addition of $Ca^{++}$ at pH 7.2 caused no change in $K_{s0.5}$ for KG and a 1.5-fold increase in $V_{max}$ whereas at pH 7.8, no change in $V_{max}$ but a 40–50% decrease in $K_{s0.5}$ and slight positive cooperative effects ("$n_{app}$" 1.4–1.7) were observed. At pH 7.8, addition of adenine nucleotides (AMP, ADP, ATP at 5mM) had no effect on the $V_{max}$; however, ATP and ADP lowered the $K_{s0.5}$ by 3.5–4-fold. The CoA derivatives of short chain fatty acids were found to inhibit the enzyme by 30–60%. This inhibition was reversed by $Ca^{++}$ but only at higher KG concentrations (250 $\mu$M). The enzyme showed a major change in activity at nicotinamide adenine dinucleotide/reduced nicotinamide adenine dinucleotide ratio (NAD/NADH) < 1, an effect that could not be modified by $Ca^{++}$. It appears, therefore, that the mussel gill KGDH is mainly regulated by changes in mitochondrial pH and ketoglutarate or adenine nucleotide levels, rather than by changes in mitochondrial calcium levels.

The metabolic regulation of the amino acids and organic acids that accumulate in hyperosmotic or anaerobically stressed estuarine bivalves involves changes in glycolysis, protein turnover, the tricaboxylic acid (TCA) cycle reactions, the transaminases and metabolite shuttling between cellular compartments (Bishop et al, '83; deZwaan, '83, Somero and Bowlus, '83). A most important aspect of these pathways is the flux of carbon through ketoglutarate (KG). These pathways include the glutamate-dependent transaminases, glutamate dehydrogenase (GDH), proline-ornithine-arginine metabolism, and the TCA cycle enzymes used in the metabolism of the five carbon intermediates (Reiss et al., '77; Greenwalt and Bishop, '80; Bishop et al., '81; DeZwaan et al., '75, '81, '82, '83; Paynter et al., '84a,b). The major TCA cycle enzyme involved in the catabolism rather than the production or recycling of KG is $\alpha$-ketoglutarate dehydrogenase (KGDH). KGDH catalyzes the formation of $CO_2$, NADH, and succinyl-CoA from KG, $NAD^+$, and CoA.

Calcium ions and adenine nucleotides but not protein phosphorylation–dephosphorylation have been shown to be the major factors regulating the KGDH activities in mitochondria from mammalian tissues (McCormack and Denton, '79; Lawlis and Roche, '80, '81 a,b; Roche and Lawlis, '82; McCormack, '85a,b). Calcium ions and adenine nucleotides also play an important role in the coordinate regulation of both the membrane transport (Pierce, '82) and the metabolic (Ellis et al., '85; Paynter et al., '85) processes

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controlling amino acid accumulation in the tissues of osmotically stressed estuarine bivalves. Therefore, we initiated this study on the possible role of $Ca^{++}$, salt, and adenine nucleotides on the regulation of the KGDH activity in ribbed mussel tissues.

Previous studies (Burcham et al., '84) have demonstrated that L-glutamate, proline, and KG stimulated a rapid rate of ADP-dependent oxygen consumption by ribbed mussel gill mitochondria, thereby providing indirect evidence for the existence of GDH, proline oxidase, and KGDH in these mitochondria and direct evidence for the tight coupling of ATP synthesis to the oxidation of these five carbon substrates. Among other invertebrates, low levels of KGDH activity have been reported in mitochondrial extracts of sea mussel tissue (Addink and Veenhof, '75), insect flight muscle mitochondria (Hansford, '72a,b; Norden and Matanganyidze, '79; McCormack and Denton, '81), and some helminth parasites (Barrett, '76). Recently, Paynter et al., ('85) have demonstrated KGDH activity in ribbed mussel gill mitochondria in a conclusive manner and have shown that the level of KGDH activity may greatly exceed (5×) the level of another important regulatory enzyme, pyruvate dehydrogenase (PDH). However, in preliminary studies, adenine nucleotide but not $Ca^{++}$ activation of insect flight muscle KGDH was demonstrated leading to the suggestion that $Ca^{++}$ may not be a regulator of KGDHs from invertebrates (Hansford, '72a; McCormack and Denton, '81).

The experiments described here indicate that the KGDH activity in ribbed mussel gill mitochondria may be regulated by changes in ADP, ATP, pH, and KG levels at low mitochondrial KG concentrations (10–300 $\mu$M) rather than by changes in mitochondrial $Ca^{++}$ levels.

## MATERIALS AND METHODS

### *Enzyme preparation*

Ribbed mussels (*Modiolus demissus*) were purchased from Northeast Marine Environmental Institute (Monument Beach, MA). The animals were maintained as described by Greenwalt and Bishop ('80). Unless otherwise noted, all reagents were purchased from Sigma Chem. Co. (St. Louis, MO) or Fisher Sci. Co. (Pittsburgh, PA).

The enzyme complex was partially purified from a lysed mitochondrial preparation according to Paynter et al., ('85). Gills were homogenized in 10 volumes of mitochondria isolation buffer (MIB) consisting of 0.4M sucrose, 20 mM HEPES, 1 mM EGTA at pH 7.5. The homogenate was filtered through Miracloth (CalBiochem), then centrifuged at 1,500g for 10 min. The resulting supernatant was centrifuged at 9,000g for 15 min, and the pellet was resuspended in the above buffer containing 0.33 mM leupeptin, 0.1 mg/ml trypsin inhibitor, 1 mM dithiothreitol, (DTT) and 20 $\mu$M rotenone. The resuspended pellet preparation (mitochondria) was sonicated and centrifuged at 20,000g for 30 min. This supernatant was centrifuged at 150,000g for 90 min and the pellet was resuspended in MIB containing 0.1 mM DTT. This preparation was cleared by centrifugation at 20,000g for 1 hr and the supernatant was used for all studies as a crude preparation of KGDH. EGTA was included in these buffer washes to remove $Ca^{++}$ (Lawlis and Roche, '80).

### *Assay of KGDH activity*

The enzyme activity was assayed spectrophotometrically in a 1-ml mixture which contained the following ingredients: 0.5 mM $NAD^+$, 0.25 mM KG, 0.1 mM CoA, 0.1 mM TPP, 0.1 mM EGTA and 1.0 mM DTT, in 150 mM HEPES, (pH 7.5). These assays were performed at 340 nm and at room temperature (22°C) in a Beckman model 3600 recording spectrophotometer. The enzyme was preincubated for 1 min with an assay medium which did not contain KG, then KG was added and the increase in absorbance at 340 nm resulting from NAD reduction was recorded. One unit of enzyme activity was defined as the amount of enzyme that would reduce a nmole of NAD per min. PDH was assayed as described previously (Paynter et al., '85).

The kinetics of the enzyme activity were studied by assaying the enzyme as described above but in 50 mM Tris-acetate at pH 7.2 and 7.8 with increasing concentrations of KG (12.5–1,500 $\mu$M). The velocity curves and the Hill plots of the enzyme were constructed as described by Segel ('75). Protein concentration was measured by the method of Lowry et al. ('51).

### *Modulators of enzyme activity*

Various modulators of enzyme activity were tested for their effect on KGDH activity. The enzyme was assayed in HEPES buffer as described above, except that the

modulators were added 2 min after the beginning of each assay and their effect on the activity was compared to the control samples with no modulator added.

*Loss of enzyme activity with storage*

Aliquots of the resuspended high speed pellet (see above) were stored for up to 1 month in various solutions consisting of one of the following ingredients: 60% sucrose, 10% glycerol, or 10% polyethylene glycol with either 100 mM potassium phosphate or 100 mM HEPES at pH 6.8, 7.5, 8.0, or 8.5. To each, 5 mM EDTA, 5 mM EGTA, 5 mM 2-mercaptoethanol, and 1% Triton X-100 were added in the presence or absence of protease inhibitors (0.34 g/l benzamidine-HCl and 0.1 g/l phenylmethane sulfonyl fluoride (PMSF). The preparations were assayed for both PDH and KGDH then stored either at −10° or at 5°C. At intervals of 3, 15, and 30 days, the stored fractions were assayed to determine the percentage of PDH and KGDH activity remaining.

*Effect of nucleotides on the activity of KGDH*

The effect of 5 mM AMP, ADP, and ATP on the activity of KGDH was assayed as described above but in 50 mM Tris-acetate, pH 7.8. The enzyme was preincubated in the presence of each nucleotide for 1 min and the assay started by the addition of KG.

*Effect of CoA derivatives on the KGDH activity*

The effect of acetyl-CoA, butyryl-CoA, propionyl-CoA, and succinyl-CoA on the activity of the enzyme was measured at 250 $\mu$M and 100 $\mu$M KG in the presence or absence of $Ca^{++}$. The enzyme was preincubated for 2 min with the CoA derivative at a final concentration of 0.1 mM and 0.5 mM, and the reaction was started by the addition of KG. $CaCl_2$ (2 mM) was added to the assay mixture 5 min later and the effect on KGDH activity was monitored for an additional 5 min.

## RESULTS

The technique used to prepare the enzyme from the gill mitochondria yielded 1,000–1,500 units of KGDH activity from about 30 g of gill tissue with a specific activity of 40 units/mg protein.

Assay of the enzymatic activity in 50–150 mM HEPES at pH 7.5, showed activity values that were double those of enzyme assayed in 100 or 200 mM Tris-Cl, Tris-acetate or potassium phosphate buffers at the same pH. Activity in 50 mM Tris-acetate was equivalent to activity in HEPES. Rotenone (20 $\mu$M) was added to the mitochondrial lysate to inactivate an indigenous NADH oxidase activity that pelleted with the KGDH activity. Rotenone at 100 $\mu$M did not affect the activity of these KGDH preparations. This preparation was free of transaminase and glutamate dehydrogenase activities.

The enzyme was reasonably stable if stored under appropriate conditions. This KGDH preparation could be stored for up to 1 month in a buffer consisting of 10% glycerol, 5 mM EDTA, 5 mM EGTA, 5 mM 2-mercaptoethanol, 1% Triton X-100, in 100 mM HEPES (pH 7.5) with a loss of only 30% activity. Enzyme stored at −10°C in buffers consisting of the above ingredients but containing 60% sucrose or 10% polyethylene glycol, in 100 mM HEPES or potassium phosphate, and enzyme stored in the glycerol plus phosphate buffer showed a significant (65–100%) decline in activity after 1 month. Fractions to which protease inhibitors (benzamidine-HCl or PMSF) were added during storage showed no change in loss of activity as compared to those fractions to which no protease inhibitors were added. Fractions stored at 5°C and at pH values higher or lower than pH 7.5 showed greater loss of activity after one month (60% for sucrose-phosphate, 70% for sucrose-HEPES, and 100% for all the others (data not shown)) as compared to those stored at −10°C and pH 7.5 for the same amount of time. PDH could not be stored under the above conditions because of the inhibitory concentration of EDTA (5 mM) included in the storage buffer.

*Kinetics*

Lawlis and Roche ('81a,b) have shown that the Km for KG with the mammalian KGDH increased considerably between pH 7 and pH 8 in the presence and absence of $Ca^{++}$. Kinetic studies of the gill KGDH at pH 7.2 and 7.8 in the presence or absence of 2 mM $Ca^{++}$ showed slightly sigmoidal, rectangular hyperbolic responses when the velocities were plotted against KG concentration (Fig. 1A,B). Addition of EGTA to 1 mM or 5 mM in these reaction mixtures in the absence of $CaCl_2$ did not change this result (Fig. 1A, B). When assayed at pH 7.2, $Ca^{++}$ addition caused only a 1.3–1.5-fold increase in $V_{max}$ and no change

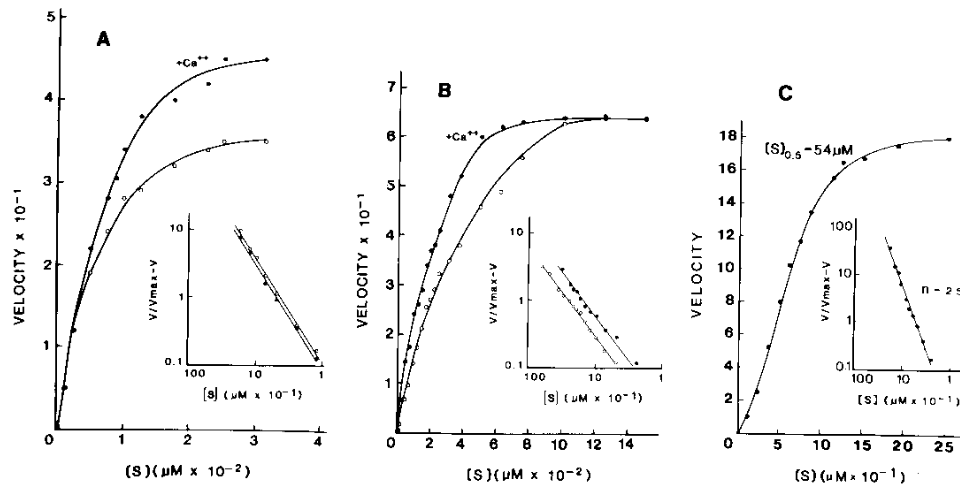


Fig. 1. Kinetic study of gill KGDH activity. Aliquots of 0.6 units of KGDH purified in the HEPES buffer system at pH 7.5 were preincubated for 1 min, in the absence of KG. Increasing concentrations of the substrate were added at 23°C in the presence (●) or absence (○) of $Ca^{++}$ and at pH 7.2(A) or 7.8(B); or KGDH purified in 3% Triton X-100 with 50 mM MOPS (pH 7.0) buffer system, assayed at pH 7.8 (1C) and the velocity recorded ($A_{340}$ $min^{-1}$).

in the $K_{s0.5}$ (50 $\mu$M) for KG (Fig. 1A). In contrast, at pH 7.8, $K_{s0.5}$ for KG was 250–300 $\mu$M and decreased slightly to 150 $\mu$M in the presence of $Ca^{++}$ with no change in $V_{max}$ (Fig. 1B). A Hill plot of these values showed an "$n_{app}$" of 1.7 at pH 7.2 and 1.4 at pH 7.8 in the presence or absence of $Ca^{++}$. None of the negative cooperative effects observed for the mammalian KGDH (Roche and Lawlis, '82) were observed with the gill KGDH.

Tissue extraction with non-ionic detergents (Triton X-100) has been used in the purification of the keto acid dehydrogenases from many mammalian tissues (Stanley and Perham, '80). Therefore, we performed kinetic studies on gill KGDH prepared in a detergent containing buffer consisting of 50 mM MOPS at pH 7.0, 2.7 mM EDTA, 0.1 mM DTT, 3% Triton X-100, 1 mM benzamidine-HCl, and 2 $\mu$M leupeptin. When activity was assayed in the Tris-acetate buffer at pH 7.8, there was a stronger positive cooperative type of behavior with a low $K_{s0.5}$ of 54 $\mu$M for KG and an "n app" value of 2.5 (Fig. 1C). Higher concentrations of Triton X-100 (5%) did not change this effect on the activity of KGDH. Because of the possible modifying effect of Triton on the KGDH activity, KGDH prepared in the absence of Triton was used in the studies reported here.

A number of other potential modulators of gill KGDH activity had only a modest effect on the KGDH activity when added at 10 mM under assay conditions of fixed, high (2.5 mM) KG concentrations (Table 1). At higher concentrations (100 mM), NaCl and KCl showed about 60% inhibition of activity whereas 100 mM sodium or potassium acetate were not inhibitory (data not shown). These results indicate that high $Cl^-$ concentrations inhibited KGDH activity. Very high concentrations of $CaCl_2$, EGTA, and $MgCl_2$ (100 mM) showed similar inhibitions of 67, 72, and 65%, respectively.

When KGDH from either mussel gills or bovine heart (Sigma Chem. Co.) was assayed at 2.5 mM KG at pH 7.8, neither showed inhibition in the presence of 1 mM EDTA (Fig. 2). This EDTA concentration was found to inhibit completely the mussel gill PDH (Paynter et al., '85). Under these conditions, $Ca^{++}$ (2.5 mM) activated the mussel gill KGDH slightly (1.5-fold) and activated the bovine heart KGDH more profoundly (5-fold). The result was in general agreement with the results of McCormack and Denton ('79,

*TABLE 1. Effect of modulators on KGDH activity from mussel gill tissue*

| Compound (10mM) | Activity (% of control) |
|---|---|
| NaCl | 100 |
| KCl | 97 |
| Sodium acetate | 100 |
| Potassium acetate | 100 |
| $CaCl_2$ | 50 |
| $MgCl_2$ | 95 |
| EDTA | 88 |
| ATP | 100 |
| ADP | 80 |
| GTP | 100 |
| GDP | 130 |
| IDP | 160 |
| AMP-PNP | 120 |
| AMP-PCP | 110 |

Mussel gill KGDH was assayed in HEPES at pH 7.5 in the presence and absence (control) of 10 mM of the indicated compounds and at 2.5 mM KG.

'81) and Roche and Lawlis ('82) for mammalian KGDH. In contrast, 2.5 mM $Mg^{++}$, which showed an activating effect similar to the $Ca^{++}$-activating effect on KGDH from bovine heart tissues, had no effect on KGDH from mussel gills (Fig. 2).

The KGDH activities of insect flight muscle and pig heart KGDH (Hansford, '72 a,b; McCormack and Denton, '79,'81) are modified by adenine nucleotides; ATP tended to inhibit and ADP or AMP tended to stimulate these enzymes by raising and lowering the $K_m$ for KG, respectively. With the gill KGDH at pH 7.8 and varying KG concentrations, 5 mM ATP, ADP, and AMP had little effect on the $V_{max}$ (Fig. 3). However, at pH 7.8, ATP and ADP lowered the $K_{s0.5}$ for KG to 60 $\mu$M and 85 $\mu$M, respectively; AMP had no effect. The sigmoidicity or "$n_{app}$" with KG changed only slightly from 1.4 to 1.7, 1.5, and 1.6 for AMP, ADP, and ATP, respectively (Fig. 3). Addition of 10 mM or 2 mM $MgCl_2$ did not modify this ATP, ADP, or AMP effect. The results of the adenine nucleotide and $Ca^{++}$ effects on the KGDH activity at pH 7.8 are summarized in Table 2.

CoA derivatives of short chain fatty acids have been shown to modify the activity of the KGDH and the other keto acid dehydrogenases (Roche and Lawlis, '82). The KGDH activity was assayed at pH 7.8 and at two low KG concentrations in order to evaluate any effect of $Ca^{++}$ on the modification of the activity by the CoA derivatives (Fig. 1B). With the gill KGDH, CoA derivatives (Fig. 4) at either 0.1 mM or 0.5 mM had similar effects on the activity when assayed in the presence and absence of 2 mM $Ca^{++}$. With 100 $\mu$M KG, $Ca^{++}$ slightly activated all preparations except those treated with butyryl CoA; all acyl-CoA derivatives but succinyl CoA were somewhat inhibitory in the absence of $Ca^{++}$. The slight activation by $Ca^{++}$ when assayed with 100 uM KG was essentially lost when the CoA derivatives were added. On the other hand, at 250 $\mu$M KG the

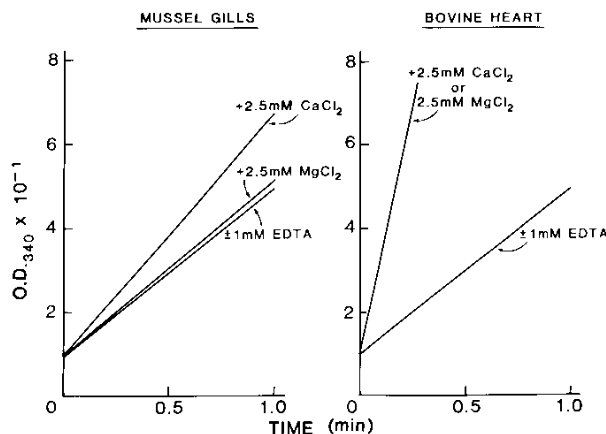


Fig. 2. Effect of $Ca^{++}$, $Mg^{++}$, and EDTA on gill and bovine KGDH activity. Aliquots of one unit of KGDH from mussel gills or bovine heart were assayed at 2.5 mM KG in the presence or absence of 2.5 mM $Ca^{++}$ or $Mg^{++}$ and 1 mM EDTA.

*TABLE 2. Effect of $Ca^{++}$, AMP, ADP, and ATP on the activity of mussel gill KGDH at pH 7.8*[1]

| | Control | $CaCl_2$ | AMP | ADP | ATP |
|---|---|---|---|---|---|
| $K_{s0.5}$($\mu$M) | 250–300 | 150 | 250 | 60 | 85 |
| $n_{app}$ | 1.4 | 1.4 | 1.7 | 1.5 | 1.6 |
| SA($V_{max}$)[2] | 40 | 40 | 40 | 40 | 36 |

[1]Data are taken from Figures 2 and 4.
[2]SA($V_{max}$), units/mg protein at maximum velocity.

activation by $Ca^{++}$ and reversal of this activation by the CoA derivatives was much less pronounced than at 100 $\mu$M KG.

The KGDH activity in mammalian tissues is inhibited by NADH accumulation and this inhibition is modified by $Ca^{++}$ (McCormack and Denton, '81; Lawlis and Roche, '80, '81a,b). Because of the modest $Ca^{++}$-activating effect on the gill KGDH (Figs. 1B, 4), it was important to determine whether or not $Ca^{++}$ would modify any NADH inhibitory effect at low (near $K_{s0.5}$) KG concentrations.

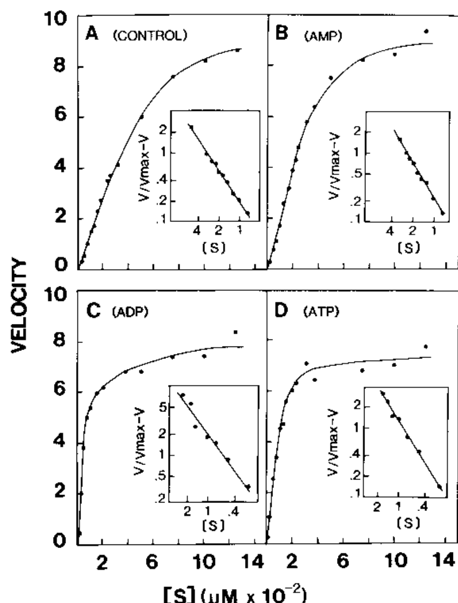


Fig. 3. Effect of nucleotides on gill KGDH activity. Aliquots of 0.5 mg of the enzyme extract were assayed in the absence (A) or the presence of 5 mM AMP (B), ADP (C), or ATP (D) at the indicated KG concentrations and the velocity recorded ($A_{340}$ $min^{-1}$ $10^2$). The Hill plots, as Log $V/V_{max}$-V vs. [S] ($\mu$M $\times$ $10^{-2}$), are shown as inserts.

At NAD concentrations of 0.05 mM, 0.1 mM, 0.5 mM, and 1.0 mM, the greatest change in the gill KGDH activity occurred at NAD/NADH ratios less than unity (Fig. 5), indicating that the KGDH complex could function maximally at NADH concentrations that would strongly inhibit the gill PDH (Paynter et al., '85). Addition of 2 mM $Ca^{++}$ at any of the NAD/NADH ratios studied did not modify this response (Fig. 5). It would appear that $Ca^{++}$ did not modify the inhibitory effect of NADH at fixed, low KG concentrations.

## DISCUSSION

The ribbed mussel gill KGDH activity appears to be less responsive to regulation by $Ca^{++}$ than the KGDH activity in mammalian tissues. With the mammalian KGDHs, $Ca^{++}$ caused a decrease in the $K_m$ for KGDH from the mM range to about 30 $\mu$M (Roche and Lawlis, '82) depending upon the pH. The response of the gill KGDH to $Ca^{++}$ is similar to that reported by McCormack and Denton ('81) for the insect flight muscle KGDH. For instance, $Ca^{++}$ addition resulted in only a small decrease (40–50%) in the $K_{s0.5}$ for KG at pH 7.8 but no change in the $K_{s0.5}$ for KG and only a slight increase in $V_{max}$ at pH 7.2 (Table 2). Added $Ca^{++}$ had only a small effect on the inhibition of the gill KGDH by acyl CoA derivatives (Fig. 4) at low KG concentrations. $Ca^{++}$ addition did not modify the inhibitory response of NADH with the gill KGDH. NADH was inhibitory only at NAD/NADH ratios below 1 or at relatively high NADH concentrations under fixed KG assay conditions (Fig. 5).

In studies by McCormack and Denton ('79) and Lawlis and Roche ('80, '81b), $Ca^{++}$ reduced the inhibitory response of the mammalian KGDH to added NADH. Because of this reduced response to NADH, the gill KGDH could probably operate in the forward direction at a reasonable rate even at the higher NADH concentrations that might occur during anaerobiosis. These results with

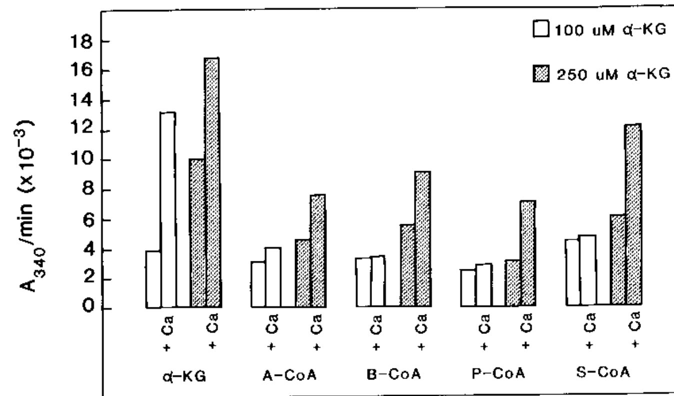


Fig. 4. Effect of CoA derivatives on the activity of gill KGDH. Aliquots of 0.5 mg of the enzyme were assayed at a concentration of 100 $\mu$M (□) and 250 $\mu$M (▨) KG in the presence of 1 mM or 5 mM acetyl-CoA (A-CoA), butyryl-CoA (B-CoA), propionyl-CoA (P-CoA), and succinyl-CoA (S-CoA) and in the presence or absence of 2 mM $Ca^{++}$.

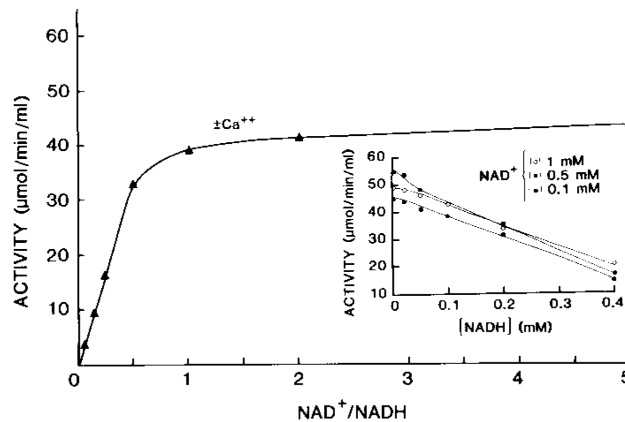


Fig. 5. Effect of NAD/NADH ratios on the activity of gill KGDH. Aliquots of the enzyme extract were assayed in 250 $\mu$M KG in the presence or absence of $CaCl_2$ (2 mM) and at various $NAD^+$/NADH ratios. KGDH was assayed at the indicated NADH concentrations and at NAD concentrations of 1 mM (○), 0.5 mM (■), 0.1 mM (●), and 0.05 mM (not shown).

the gill KGDH strongly support the suggestion by McCormack and Denton ('81) that KGDHs from invertebrate tissues are considerably less responsive to changes in mitochondrial $Ca^{++}$ levels than KGDHs from vertebrate tissues.

Recently, McCormack and Denton ('82) have suggested that $Ca^{++}$ may not be an important regulator of mitochondrial metabolism in invertebrates because of lack of KGDH $Ca^{++}$ sensitivity and the presence of a high $K_T$, ruthenium red-insensitive $Ca^{++}$ transporter in some invertebrate mitochondria. If this is so, then marine invertebrates, such as marine bivalves, with high intracellular $Na^{++}$ levels (Pierce, '82) would have an additional $Ca^{++}$ regulatory problem because the mitochondrial $Na^+/Ca^{++}$ antiporter

would act to override any regulatory action of the $Ca^{++}$ transporter. Although these limited data do not permit exclusion of $Ca^{++}$ as a regulator of metabolism in marine invertebrate mitochondria, it is evident that there are some profound differences between vertebrates and invertebrates in both the performance of some key regulatory enzymes and the ability to control mitochondrial $Ca^{++}$ levels.

The effect of lower pH on the lowering of the $K_{s0.5}$ for KG with gill KGDH (Figs. 1A,B) is similar to that reported for the mammalian KGDHs (Roche and Lawlis, '82). Both ADP and ATP caused a marked activation of the gill KGDH activity by lowering the apparent $K_{s0.5}$ for KG from 250–300 $\mu$M to 60 $\mu$M and 85 $\mu$M, respectively, when assayed at pH 7.8 (Table 2). The ADP activating effect is similar to that reported for the mammalian KGDHs (Lawlis and Roche, '81a; McCormack and Denton, '79) and insect flight muscle KGDH (Hansford, '72a,b; McCormack and Denton, '81). However, the activation rather than inhibition of the gill KGDH by ATP as reported for the mammalian and insect KGDHs means that the gill KGDH may differ somewhat from the other KGDHs. This ATP-ADP activating effect was $Mg^{++}$ independent, thereby negating any complicating effect of adenylate kinase. It would appear that the gill KGDH is regulated by changing pH and adenine nucleotide (ADP and ATP) levels and by the ability of the mitochondrion to concentrate KG or to provide reasonable KG levels (10–300 $\mu$M) with the mitochondrion.

Studies on ADP dependent respiration by mitochondria from a number of bivalves (Zaba, '78; Burcham et al., '83, '84; Ballantyne and Storey, '83; Ballantyne and Moon, '85; Moyes et al., '85) indicate that the rate of $O_2$ consumption by glutamate and KG is essentially identical when supplied with substrate in the 1–10 mM range. Therefore, KG and glutamate can be transported into and possibly concentrated within respiring bivalve tissue mitochondria. Although the concentration of KG in bivalve tissue mitochondria is not known, the estimated KG concentration in mammalian heart mitochondria (Williamson et al., '72; LaNoue and Schoolwerth, '79) can vary between 30 and 400 $\mu$M depending upon the respiratory state and pumping abilities of the mitochondria. Bayne ('73) estimated that ketoglutarate concentrations in sea mussel tissues are in the 2–15 $\mu$Mol/kg wet wt range. Because the performance of the bivalve tissue mitochondria and mammalian mitochondria are similar (see references cited above), one can reasonably assume that abilities of gill mitochondria to concentrate KG should be similar to those of mammalian mitochondria. Given this assumption, the level of KG within the mitochondria should vary in a concentration range of 30–300 $\mu$M or below the $K_{s0.5}$ for KG with the KGDH at pH 7.8 but far above or near the $K_{s0.5}$ for KG at pH 7.2. Lowering the pH to 7.2 or increasing the ADP or ATP levels would provide a strong activating effect on the KGDH activity at KG concentrations between 10 and 300 $\mu$M. Therefore it would appear that the flux of metabolites through the gill KGDH reaction would be regulated by the respiratory state of the mitochondrion and the availability of substrate to the mitochondrion.

As a corollary, the isocitrate dehydrogenase (ICDH) from insect flight muscle differs from the mammalian ICDH by not being activated by $Ca^{++}$ (McCormack and Denton, '81). Both the $NAD^+$- and $NADP^+$-dependent ICDH's are present in bivalve tissues (Addink and Veenhof, '75; Head and Gabbot, '80; Kargbo and Swift, '83; Ruiz et al., '85b). Although Kargbo and Swift ('83) showed ADP activation of the oyster $NAD^+$-dependent mitochondrial ICDH, there is no data on the role of $Ca^{++}$. These studies on ICDH and this report on KGDH combined with those on the activation of GDH by ADP (Addink and Veenhof, '75; Reiss et al., '77; Ruiz et al., '85a) mean that the three mitochondrial dehydrogenases utilizing or producing KG in bivalves are probably regulated by varying $H^+$, adenine nucleotide, and substrate levels rather than by varying $Ca^{++}$ levels within the mitochondria.

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