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SUCCINATE DEHYDROGENASE IN VARIOUS TISSUES OF *ANODONTA COUPERIANA*, *ELLIPTIO BUCKLEYI* AND *MERCENARIA CAMPECHIENSIS* (MOLLUSCA: BIVALVIA)

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Abstract—1. The specific activities, kinetic constants and pH optima for succinate dehydrogenase were determined in mantle, gill and adductor muscle from three bivalves (*Anodonta couperiana*, *Elliptio buckleyi* and *Mercenaria campechiensis*).

2. In general the K_m values for succinate and K_i values for fumarate differed in all bivalve tissues whereas the K_i for malonate was consistently low.

3. The ratio of K_i (fumarate) to K_m (succinate) was less than one for all tissues studied. This is similar to many facultative anaerobes with fumarate reductase activity.

INTRODUCTION

Succinate is one of the major end products of metabolism in many facultative anaerobes such as microbes (Hirsh *et al.*, 1963), parasitic helminths (Saz, 1971) and bivalve molluscs (Holwerda and de Zwaan, 1979, 1980). The enzyme succinate dehydrogenase (SDH) is presumed to catalyze, under anaerobic conditions, the NADH dependent reduction of fumarate to succinate, thus acting as a fumarate reductase (Singer *et al.*, 1972). This is an apparent reverse reaction of the aerobic succinate to fumarate oxidative step in the tricarboxylic acid cycle. Thus, fumarate would act as the electron acceptor in the absence of oxygen.

Most studies of anaerobic metabolism in molluscs and parasitic helminths have investigated concentrations of suspected end products (Hammen and Wilbur, 1959; Stokes and Awapara, 1968; Gade, 1975). Many investigators have not looked at the kinetic properties of SDH, but, instead, have investigated its presumed fumarate reductase properties (Hammen and Lum, 1966; Wegener *et al.*, 1969; Hammen, 1975; Holwerda and de Zwann, 1979). For example, in *Escherichia coli*, two types of SDH occur which are inducible depending on environmental conditions (Ruiz-Herrera and Garcia, 1972). In contrast, in the parasitic nematode *Ascaris lumbricoides* one enzyme seems to act in both capacities; the direction of catalysis depending on the stage in the life cycle (Fairbairn, 1970). Intertidal marine and freshwater molluscs may also have the ability to adjust their metabolism to environmental demands by the modulation of SDH.

This study investigated the kinetic properties of SDH as it catalyzes the oxidation of succinate to fumarate in three bivalve molluscs; *Anodonta couperiana* and *Elliptio buckleyi* (freshwater clams), and *Mercenaria campechiensis* (marine southern quahog). Kinetic constants were determined for several substrates and inhibitors for possible correlation with succinate or fumarate metabolism.

MATERIALS AND METHODS

Collection and maintenance of animals

Anodonta couperiana (Lea) and *Elliptio buckleyi* (Lea) were maintained in aerated well water with their natural substrata. Clams were used within 24 hr to two weeks after collection. *Mercenaria campechiensis* (Gmelin) were maintained in artificial seawater (25‰) in a recirculating 100 gal. aquarium with natural substratum and were also used 24 hr–2 weeks after collection.

Preparation of tissues

The clams *E. buckleyi* and *A. couperiana* were opened and their gill, adductor muscle and mantle tissues excised and weighed. These tissues were washed once in chilled 0.04 M Tris–HCl buffer, pH 7.4, containing 0.25 M sucrose and 1.0 mM EDTA. Gill, adductor muscle and mantle tissues of *M. campechiensis* were excised, weighed and washed once in chilled 0.04 M HEPES buffer, pH 7.4, containing 0.25 M sucrose, 2.0 mM EDTA, 0.25 M mannitol and 2.0 mM EGTA (Holwerda and de Zwaan, 1979). All tissues were homogenized in a Waring blender by three 5 sec bursts with 10 sec 'off' intervals in their chilled buffers. These crude homogenates were diluted 9 × (w:v).

Preparation of mitochondrial extract

The crude homogenates were centrifuged at 1200 × ***g*** for 10 min (4°C). The resulting supernates were decanted and recentrifuged for 20 min at 12,000 × ***g***, 4°C. The resulting pellets were resuspended in their respective buffers using a Ten Broeck homogenizer, then recentrifuged twice at 20,000 × ***g*** for 20 min (4°C). The final washed pellets were resuspended in their respective buffers. All pellets and supernatants were assayed for SDH activity and protein.

Enzyme assay of SDH

A modification of the method of Arrigoni and Singer (1962) was used for the determination of SDH activity. The change in optical density of 2,6-dichlorophenol indolphenol (DCPP) at 610 mn is measured. The reaction mixture contained: 0.055 M glycylglycine buffer, pH 7.9; 0.1 mM DCPP; 0.045% BSA; 0.08% phenazine methosulfate; 0.23 mM KCN and 20.0 mM Na succinate in 1 ml total vol. The reaction was started with the addition of the enzyme preparation and compared to a control which lacked substrate (succinate).

Table 1. A comparison of specific activities, pH and kinetic constants of succinate dehydrogenase in mantle, gill and adductor muscle of *Anodonta couperiana*

Preparation	Sp. act. (μmoles/min/mg protein)	pH	Kinetic constants $K_m \times 10^{-3}$ M Succinate	$K_i \times 10^{-3}$ M Fumarate	Malonate
Mantle	0.072	8.0	0.48	0.17	0.006
Gill	0.071	8.0	2.00	0.20	0.08
Adductor muscle	0.014	8.0	25.50	5.00	1.69

pH profiles

For pH profiles, 0.020 M HEPES and 0.020 M MES buffers were used. Succinate concentration was kept at 20 mM and 50–100 μl of homogenate was used.

Kinetic constants

Michaelis constants (K_m) and inhibition constants (K_i) were evaluated using double reciprocal plots. Malonate and fumarate inhibition constants were determined by using fixed concentrations of the inhibitor in differing succinate concentrations, as well as different concentrations of the inhibitor in a fixed succinate concentration. Each determination was replicated 3–5 times over 5–7 experimental concentrations. Data was subjected to linear regression analysis and each apparent kinetic constant was calculated as a mean of 2–3 trials.

Specific activities

Specific activities were determined using a molar extinction coefficient of 2.1×10^{-4}/M cm for DCPP (King, 1963) and are expressed as μmoles succinate reacted/min/mg protein. Protein was determined by the method of Lowry *et al.* (1951).

Reagents

Assays were performed using a Beckman Model 24 Spectrophotometer with chart recorder. Assay reagents were purchased for Sigma Chemical Co.

RESULTS

Anodonta couperiana

Apparent specific activities of SDH adductor muscle, mantle and gill tissues of *A. couperiana* are listed in Table 1. Mantle and gill have similar apparent specific activities, while the adductor muscle value is 5 × less.

The pH optima for all three tissues of *A. couperiana* were the same (Table 1). Hydrogen ion concentrations had a pronounced effect on the apparent specific activities of SDH of mantle and gill tissue, but not of adductor muscle (Fig. 1). The Michaelis constants for succinate for the tissues of *A. couperiana* differed widely, ranging from 0.48–255 mM (Table 1). Also, widely differing inhibition constants for fumarate were found for all three tissues (Table 1), but all values are less than the K_m for succinate. In all three tissues a low concentration of malonate inhibited SDH activity and again, K_i values for malonate are much less than the K_m values for succinate (Table 1). In all cases, the inhibition by fumarate and malonate appeared to be competitive.

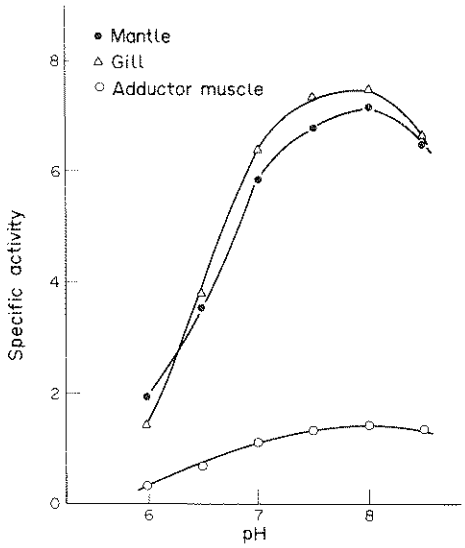

Fig. 1. Effect of pH on the sp. act. (μmoles succinate converted/min/mg protein × 10^{-2}) of succinate dehydrogenase of mantle, gill and adductor muscle of *Anodonta couperiana*.

Elliptio buckleyi

Apparent specific activities of SDH for mantle, gill and adductor muscle tissues of *Elliptio buckleyi* are similar (Table 2).

The optimal pH for SDH for all three tissues of *E. buckleyi* was 8.0 (Table 1), and the pH curves for gill and adductor muscle peak sharply at pH 8.0 (Fig. 2). In contrast, the pH curve for mantle is smooth.

Michaelis constants for succinate for all three tissues are more similar than for tissues of *A. couperiana* and range from 0.10–0.32 mM (Table 2). Low concentrations of both fumarate and malonate inhibited the SDH activities in all three tissues (Table 2). Very low K_i values for both fumarate and malonate were found. In all cases, inhibition was competitive.

Mercenaria campechiensis

Apparent specific activities of SDH for adductor muscle, mantle and all tissues of *M. campechiensis* are listed in Table 3. Mantle and gill tissues have similar specific activities (0.070 and 0.074 μmoles/min/mg protein, respectively), while the adductor muscle value is 5 × less (0.17).

Table 2. A comparison of specific activities, pH and kinetic constants of succinate dehydrogenase in mantle, gill and adductor muscle of *Elliptio buckleyi*

Preparation	Sp. act. (μmoles/min/mg protein)	pH	Kinetic constants $K_m \times 10^{-3}$ M Succinate	$K_i \times 10^{-3}$ M Fumarate	Malonate
Mantle	0.038	8.0	0.18	0.064	0.026
Gill	0.039	8.0	0.32	0.032	0.026
Adductor muscle	0.050	8.0	0.10	0.030	0.09

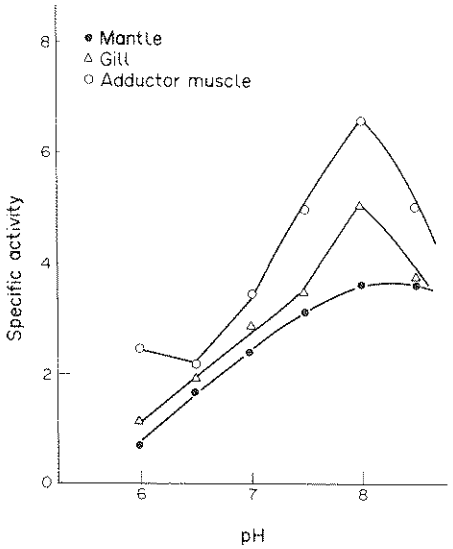

Fig. 2. Effect of pH on the specific activities (μmoles succinate converted/min/mg protein $\times 10^{-2}$) of succinate dehydrogenase of mantle, gill and adductor muscle of *Elliptio buckleyi*.

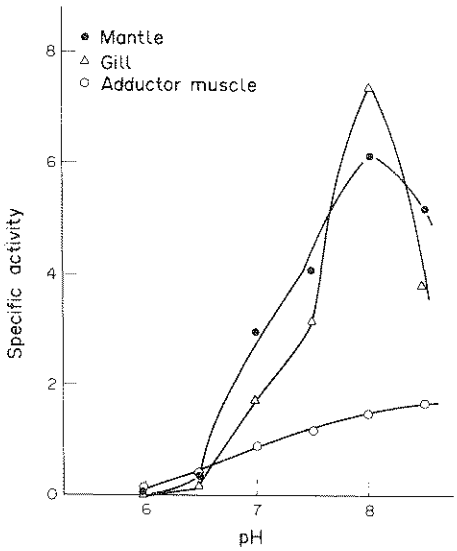

Fig. 3. Effect of pH on the specific activities (μmoles succinate converted/min/mg protein $\times 10^{-2}$) of succinate dehydrogenase of mantle, gill and adductor muscle of *Mercenaria campechiensis*.

The pH curves of the mantle and gill peak sharply at pH 8.0, while the adductor muscle smoothly rises to pH 8.5 (Fig. 3).

The K_m values (Table 3) for succinate are 1.0, 3.75 and 4.0 mM for gill, adductor muscle and mantle tissues, respectively. Low concentrations of fumarate inhibited SDH activity in both the mantle and gill tissues, with the K_i values (Table 3) much lower than the K_m values for succinate for these two tissues. In contrast, the K_i for fumarate is close to the K_m value for succinate in the case of the adductor muscle. For all tissues, low malonate concentrations inhibited SDH activity and the K_i values for malonate are much lower than the K_m values for succinate. In all cases, inhibition by fumarate and malonate were competitive.

DISCUSSION

Under anaerobic conditions carbohydrates are thought to be the primary or only source of energy in bivalve molluscs (de Zwaan and Wijseman, 1976; de Zwaan *et al.*, 1976; Kluytmans *et al.*, 1978). Fumarate has been postulated (de Zwaan and Wijseman, 1976) and shown (Hammen, 1975; Holwerda and de Zwaan, 1979) to be the electron acceptor in a series of reactions similar to those seen in facultative anaerobic bacteria (Kroger, 1978; Hirsch *et al.*, 1963) and parasitic helminths (Saz, 1971; Kohler and Bachman, 1980). Under these conditions the reversal of SDH activity facilitates the sinking of electrons; their coupling to ATP production is apparently more efficient than anaerobic pathways utilizing LDH (de Zwaan *et al.*, 1976). In *Mytilus edulis*, phosphoenolpyruvate carboxykinase activity favors these reactions under anaerobic conditions (de Zwaan, 1977).

Apparent specific activities

In general the apparent specific activities for SDH in various animals are thought to decrease as their environments become more anaerobic (Table 4). In this study, the apparent SDH activities of the tissues of the bivalves fall within the anaerobic range. Except for the adductor muscles of *A. couperiana* and *M. campechiensis* the specific activities for all other tissues are very close to the values obtained by Hammen and co-workers (Wegener *et al.*, 1969; Hammen, 1969). In adductor muscles of both *A. couperiana* and *M. campechiensis*, the specific activities were much lower than those seen for the gill and mantle. These lower values may have been due to interference from the large amount of glycogen present in these tissues.

pH profiles

The pH optima for SDH of all tissues of the bivalves studied are similar with the exception of adductor muscle tissue SDH of *M. campechiensis*. The pH range for optimal SDH activity is 7.4 to 8.0 (Table 5). In contrast, the shapes of the curves differed among the bivalves studied and also among their tissues. This may be indicative of differing

Table 3. A comparison of specific activities, pH and kinetic constants of succinate dehydrogenase in mantle, gill and adductor muscle of *Mercenaria campechiensis*

Preparation	Sp. act. (μmoles/min/mg protein)	pH	Kinetic constants $K_m \times 10^{-3}$ M Succinate	$K_i \times 10^{-3}$ M Fumarate	$K_i \times 10^{-3}$ M Malonate
Mantle	0.070	8.0	4.00	0.12	0.19
Gill	0.074	8.0	1.00	0.41	0.48
Adductor muscle	0.017	—	3.75	3.52	0.64

Table 4. Specific activities (μmoles/min/mg protein) of succinate dehydrogenase from various organisms

Organism or tissue	Specific activity	Reference
Anaerobes		
Micrococcus lactilyticus	0.0315*	Singer (1971)
Proteus vulgaris	0.0200†	Singer (1971)
Saccharomyces cerevisiae	0.0070†	Singer (1965)
Temporary anaerobes		
Crassostrea virginica		
mantle	0.0149†	Wegener *et al.*
muscle	0.0050†	(1969)
Mercenaria mercenaria		
mantle	0.0820†	Hammen (1969)
muscle	0.0800*†	
heart	0.0800*†	
Modiolus demissus		
mantle	0.0390†	Hammen (1969)
muscle	0.1100†	
Anodonta couperiana		
mantle	0.0720†	Present study
gill	0.0710†	
adductor muscle	0.0140†	
Elliptio buckleyi		
mantle	0.0380†	Present study
gill	0.0390†	
adductor muscle	0.0500†	
Mercenaria campechiensis		
mantle	0.0700†	Present study
gill	0.0740†	
adductor muscle	0.0170†	
Aerobes		
Saccharomyces cerevisiae	1.5000†	Singer (1965)
Beef heart	1.26–1.97*	Singer (1971)
Rat liver	0.2480†	Rodrick (unpublished)

*Oxygen consumed.
†Succinate converted.

effects on SDH related reaction sequences and may also reflect an influence of pH in the external environment.

Kinetic constants

The kinetic constants, K_m (succinate), K_i (fumarate) and K_i (malonate) for SDH differ in each tissue for each bivalve. Michaelis constants for succinate ranged from what is considered representative for aerobic activity (Singer, 1971) (Table 4) to outside a normal physiological range for the tissue (25 mM succinate) (Zurburg and Kluytmans, 1980). A comparison of these constants to others (Holwerda and de Zwaan, 1979; 1980; Hammen, 1975) is difficult since most studies have looked at one tissue. These data have been used to explain the metabolic patterns of the whole organism. Studies which have determined the incorporation of labelled compounds into succinate or alternatively, anaerobic accumulation of succinate have shown that different bivalve tissues give different rates of incorporation and likewise accumulate succinate at differing rates (Chaplin and Loxton, 1976; Zurburg and Kluytmans, 1980). However, these types of data do not give any indication how the SDH might be involved in aerobic/anaerobic mechanisms.

The SDH of some of the molluscan tissues in this study (e.g., mantle, gill and adductor muscle of *E. buckleyi*, mantle of *A. couperiana* and gill of *M. campechiensis*) show both low K_m values for succinate and low K_i values for fumarate. The SDH of aerobic organisms (Table 5), in contrast, have greater K_i values for fumarate than K_m values for succinate. Therefore, in these molluscan tissues under appropriate conditions, SDH may function in the oxidation of succinate as a 'typical' TCA cycle enzyme. In these cases, adaptation of this enzyme to efficient fumarate reduction could be restricted by a higher affinity for succinate in the oxidative direction. In fact, the ratio of K_i (fumarate) to K_m (succinate) as an index for aerobic organisms is seen to be greater than one, while the ratio for anaerobic and facultative anaerobes is much less than one (Table 6). Therefore, in all of the above molluscan tissues, these ratios suggest that very low concentrations of fumarate would be expected to inhibit the forward oxidative SDH reaction and the enzyme acts in the reverse direction under anaerobic conditions. It has been suggested that for species which lack oxygen carriers, the respiratory role of the blood is so small that anaerobic pathways exist in deep tissues even under aerobic conditions (Dykens and Magnum, 1978; Stokes and Awapara, 1968). Although the SDH of the adductor muscle of *M. campechiensis* has a K_m within the range of the representative temporary anaerobes (Table 5), the K_i for fumarate suggests that, compared to the previous tissues, somewhat higher concentrations of fumarate are needed to inhibit the forward reaction. The adductor muscle of *A. couperiana* shows an extremely high K_m for succinate, possibly out of its physiological range and it is not clear how such a high apparent K_m would allow *in vivo* activity of the enzyme. However, Zurburg and Kluytmans (1980) found that the mantle and gill of *Mytilus edulis* show faster rates of anaerobic succinate accumulation than adductor muscle. The differences seen in SDH characteristics may actually be indicative of biochemical tissue diversity in the organisms.

Table 5. Kinetic constants of succinate dehydrogenase from various organisms

Organism	Preparation	Assay conditions* pH (opt.) (PMS-DCPP)	Temp (°C)	K_m (mM) Succinate	K_i (mM) Fumarate	Malonate	Reference
I. Aerobes							
Escherichia coli	aerobic, normal	7.5	30	0.26			Singer (1971)
	homogeneous						
Beef heart	homogeneous enzyme	7.6	38	1.3	1.9	0.041	Singer *et al.* (1956)
			25	0.52	0.8	0.025	
Pumpkin seedlings	purified enzyme	7.8	35	1.4	1.5	0.061	Singer (1971)
Proteus vulgaris	aerobically grown	7.6	38	1.3	1.8		Kearney and Singer (1956)
Saccharomyces cerevisiae	homogeneous enzyme	7.8	38	1.0	1.03	0.0105	Hauber and Singer (1967)
II. Facultative anaerobes							
Mytilus edulis	purified adductor muscle	7.7	35	2.0	0.15	0.06	Ryan and King (1962)
Mercenaria mercenaria	600 × *g* supernate	7.9	25	30.0			Hammen (1975)
	adductor muscle						
Mytilus edulis	20,000 × *g* pellet mantle	7.9	25	0.1			Holwerda and de Zwaan (1979)
Propionibacter pentosaceum	purified enzyme	7.4	30	2.2			Singer (1971)
Escherichia coli	mutant (S-)	7.5	30	1.0			Singer (1971)
III. Obligate anaerobes							
Claviceps purpurea	purified enzyme	7.7	35	3.3	0.93	0.03	Singer (1971)
Micrococcus lactilyticus	homogeneous enzyme	7.6	30	5.3	0.22	0.23	Singer (1971)

Table 6. Ratio of the K_i (fumarate) to the K_m (succinate) of succinate dehydrogenase from various organisms

Animal, tissue	K_i/K_m
Micrococcus lactilyticus	0.04
Claviceps purpurea	0.28
Mytilus edulis, adductor muscle	0.075
Beef heart	1.46
	1.53
Proteus vulgaris	1.38
Pumpkin seedlings	1.07
Saccharomyces cerevisiae	1.03
Anodonta couperiana, mantle	0.375
Anodonta couperiana, gill	0.100
Anodonta couperiana, adductor muscle	0.196
Elliptio buckleyi, mantle	0.035
Elliptio buckleyi, gill	0.100
Elliptio buckleyi, adductor muscle	0.300
Mercenaria campechiensis, mantle	0.030
Mercenaria campechiensis, gill	0.410
Mercenaria campechiensis, adductor muscle	0.940

Based on the kinetic data of the forward reaction, the SDH of the mantle, gill and adductor muscle tissues of *Elliptio buckleyi* and the mantle and gill tissues of *Mercenaria campechiensis* and *Anodonta couperiana* could participate in proposed anaerobic pathways which show succinate as an end product (de Zwaan *et al.*, 1976; Holwerda and de Zwaan, 1980). The low K_m values for the tissues of *E. buckleyi*, mantle of *M. campechiensis* and gill of *A. couperiana* suggest that the SDH will function in the TCA cycle. The SDH of the adductor muscle tissue of both *M. campechiensis* and *A. couperiana* gave less interpretable kinetic data, possibly indicating either problems with preparation (high glycogen content) or that the control of SDH is not as important in these tissues. In general, the low apparent specific activities of the three tissues of the three bivalves resemble those found for other temporary anaerobes.

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