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SUBCELLULAR LOCALIZATION AND CHARACTERIZATION OF HCO_3^--ATPASE FROM THE MANTLE OF THE FRESHWATER CLAM, *ANODONTA CATARACTA*

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Abstract—1. HCO_3^--stimulated ATPase activity was demonstrated in mantle tissue of the freshwater clam, *Anodonta cataracta*.

2. Calcium (1 mM) slightly inhibited and SCN^- completely inhibited HCO_3^--stimulation of the enzyme.

3. ATPase activity had a K_m of 6.8 mM for HCO_3^--activation and was inhibited at HCO_3^--concentrations > 20 mM.

4. Subcellular fractionation studies revealed the presence of both a mitochondrial and a non-mitochondrial HCO_3^--ATPase.

INTRODUCTION

A bicarbonate-stimulated ATPase has been described in many mammalian tissues (for examples, see Table 3). The enzyme has also been described in some lower vertebrates including the frog (Grisola & Mendelson, 1974); teleost fish (DeRenzis & Bornancin, 1977; Kerstetter & Kirscher, 1974; Ho & Chan, 1981); and *Necturus* (Wiebelhaus *et al.*, 1971). However, very little is known about such anion ATPase in invertebrates. A recent paper by DePew & Towle describes and characterizes some properties of the enzyme in fiddler crab gill (DePew & Towle, 1979), and Wheeler (1975) presents evidence for the presence of a HCO_3^--ATPase in the mantle epithelium of the American oyster. In the present paper, the study of the enzyme has been extended to a freshwater invertebrate, the clam *Anodonta cataracta*.

In addition to identifying the enzyme and characterzing some of its kinetic properties, an attempt was made to localize the HCO_3^--ATPase following subcellular fractionation. Previous fractionation studies on this enzyme suggest its location in the mitochondrial or microsomal fractions. In many systems, HCO_3^--ATPase (DePew & Towle, 1979). Using differexamples, see Table 3). The earlier evidence favored an exclusively mitochondrial location for this enzyme, (Van Amelsvoort *et al.*, 1977a,b); however, there is growing evidence for a plasma membrane bound HCO_3^--ATPase (DePew & Towle, 1979). Using differential and isopycnic zonal centrifugation methods, an attempt was made in the present study to identify the subcellular location(s) of a HCO_3^--ATPase in the clam mantle epithelium.

The mantle was chosen for this study because this tissue is responsible for secreting the largely $CaCO_3$ shell of molluscs (Wilbur, 1964). A bicarbonate pump may be partially responsible for transporting a supply of carbonate precursors from the blood or medium to the extrapallial fluid, the compartment in which the shell forms. Wheeler *et al.* (1975) have demonstrated that carbonate precursors from the medium represent the major source of shell carbonate in the Bay Scallop (*Argopecten irradians*). Because at the pH of seawater most of the dissolved inorganic carbon is in the form of bicarbonate, transport of this ion across scallop mantle seems plausible. Evidence has also been provided that active transport of HCO_3^- may occur in the isolated mantles of the oyster, *Crassostrea virginica* (Wheeler, 1975, 1980), a tissue in which a HCO_3^--ATPase has been located (Wheeler, 1975).

MATERIALS AND METHODS

Collection and homogenization

Freshwater clams (*Anodonta cataracta*), collected from Lake Hartwell, SC, were kept in tanks of aerated lake water at 18–20°C. They were used within 2 weeks following collection.

Whole clam mantles (5 g) were washed and then homogenized in 20 ml ice cold 0.25 M sucrose containing 5 mM HEPES† at pH 7.0. The homogenization was performed in a two-stage process using a motor driven homogenizer (860 rev/min). The first stage consisted of a 2–3 min homogenization with a ground-glass pestle, followed by filtration of the homogenate through two layers of cheesecloth. The second stage consisted of 10 strokes with a teflon pestle. The homogenate was then immediately assayed for enzyme activity or fractionated.

Differential centrifugation

Differential rate centrifugation was routinely performed using the following sequences: 1085 × g for 10 min (nuclear pellet), 14,000 × g for 20 min (mitochondrial pellet), and 100,000 × g for 60 min (microsomal pellet and final supernatant). All fractions were observed with a phase contrast microscope to check for the consistency of particle separations.

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† N-2-hydroxyethyl piperazine-N^1-2-ethane sulfonic acid.

In an attempt to produce a pellet with a high percentage of mitochondria and a measurably higher cytochrome oxidase activity than that of the previous fractions, the following modified sequences were used: 300 × *g* for 10 min to produce a heavy nuclear pellet; 1935 × *g* for 20 min to produce a heavy mitochondrial pellet; and 14,500 ± *g* for 20 min to produce a light mitochondrial pellet.

All pellets were resuspended by gentle homogenization in homogenizing medium containing 1% Triton X-100. Samples to be assayed for cytochrome oxidase were not treated with Triton because the detergent inhibited the activity of this enzyme.

Zonal centrifugation

To prepare the homogenate fractions for isopycnic zonal centrifugation, the following fractionation procedure was used: two successive centrifugations at 600 × *g* for 15 min, discarding the resulting pellets, and recentrifugation of the supernatant at 105,000 × *g* for 40 min. The resulting pellet was resuspended in homogenization medium and 20 ml were loaded on a discontinuous gradient consisting of the following sucrose solutions (w:w): 150 ml, 15%; 150 ml, 35%; and 200 ml, 50%. Enough 55% sucrose cushion and buffered overlay (1 mM HEPES) was included to fill the rotor. All sucrose solutions were buffered with 1 mM HEPES. The sample was spun in a Ti-14 zonal (Beckman) at 45,000 rev/min for 60 min and collected in 15 ml fractions by displacement of the gradient with 55% sucrose using a gradient pump. The OD of the effluent was monitored continuously at 250 nm.

In order to subfractionate the mitochondrial fraction, a rate zonal method was used. Two successive centrifugations were made at 600 × *g* for 10 min and the resulting pellets were discarded. A final centrifugation was performed at 14,500 × *g* for 20 min. The resulting pellet was resuspended in homogenization medium and 2–3 ml were introduced under a buffered overlay in a centrifuge tube and were displaced with a linear with volume sucrose gradient (24 ml, 15–30%). A 50% sucrose cushion was added to fill the tube. The fraction was centrifuged at 12,000 × *g* for 10 min in a type 30 rotor (Beckman). Approximately 3 ml fractions were collected from a hole punctured in the bottom of the centrifuge tube.

Enzyme assays

For Mg^{2+}-ATPase the assay medium contained 6 mM $MgSO_4$, 50 mM HEPES (pH 8.0), and 3 mM Tris or disodium ATP (Sigma) in a 1.0 ml volume. This medium, with the addition of 10 mM $NaHCO_3$, was used to assay for HCO_3^--ATPase, or, with the addition of 50 mM KCl, was used to assay for Na^+, K^+-ATPase. The effect of 1 mM $CaSO_4$ on ATPase activity was tested in both the Mg^{2+}-ATPase and HCO_3^--ATPase assay media. To test for activation of ATPase due to Cl^- and SO_4^{2-}, these anions were added as 10 mM NaCl or Na_2SO_4 to the Mg^{2+}-ATPase assay medium. The effect on ATPase activity of the anion ATPase inhibitor, SCN^-, and the Na^+, K^+-ATPase inhibitor, ouabain, was determined by addition of the inhibitor (10 mM) to various assay media.

For all ATPase activities the filtered homogenate or subcellular fraction (0.1 ml) was incubated for 60 min at 23–25°C. The reaction was initiated by the addition of ATP and completed by the addition of 1 ml, 3% TCA. Spectrophotometric blanks were prepared in the same manner as for the enzyme assay with the exception that the tissue sample was added after the acid. Phosphate analysis was accomplished by the following procedure, modified after Marsh (1959): 2 ml butanol followed by 0.2 ml acid molybdate solution (5 g ammonium molybdate and 22 ml H_2SO_4 to 100 ml) were added to the test solution. After vortexing for 15 sec, the solution was neutralized by a 0.5 ml citrate solution (100 g/500 ml, pH 6.5–7.5) and again vortexed for 15 sec. The solution was then centrifuged to clearly separate the butanol phase. The absorbance of the butanol phase was read at 350 or 400 nm. Standards of orthophosphate in the range of 0.1 to 2.0 μmol were prepared in 1 ml volumes and treated in the same manner as the assay media. Enzyme activity was expressed in μmol orthophosphate liberated per hour.

Enzyme activation is here defined as the per cent increase of the rate of ATP hydrolysis over the hydrolysis when the only exogenous inorganic ions are supplied as 6 mM $MgSO_4$, present in all the assay media. Total activity is the enzyme activity when other ions in addition to 6 mM $MgSO_4$ are present in the assay media. Therefore, HCO_3^--ATPase activity is the total activity with $NaHCO_3$ (usually 10 mM) in the assay media minus the activity due to 6 mM $MgSO_4$ alone in the assay media.

Cytochrome oxidase was assayed in a medium containing 0.1 ml of a 1% cytochrome c solution (reduced by sodium dithionate), 50 mM HEPES (pH 8.0), and 0.2 ml sample in a final volume of 2.0 ml. The addition of reduced cytochrome c initiated the reaction, and the rate of cytochrome oxidation was measured as the change in absorbance per minute.

Alkaline and acid phosphatase were assayed in a 2.0 ml volume containing either 50 mM sodium succinate (pH 5.0) for acid phosphatase, or 50 mM sodium glycinate (pH 10.0) for alkaline phosphatase, along with 0.1 ml sample. The reaction was initiated by the addition of 5 mM *p*-nitrophenylphosphate (Sigma), run for 30 min and terminated by 0.2 ml, 1 N NaOH. The samples were centrifuged if necessary, and the absorbance of *p*-nitrophenol liberated was determined at 400 nm. Spectrophotometric blanks were prepared in the same fashion as the routine assays with the exception that the tissue sample was added after the NaOH. Enzyme activity was expressed in μmol *p*-nitrophenol liberated per minute.

Kinetics

To obtain material with the highest specific activity (activity/μg protein) of HCO_3^--ATPase, the following fractionation procedure was employed: two successive centrifugations were performed at 900 × *g* for 10 min and the resulting pellets were discarded. The residual material was centrifuged at 100,000 × *g* for 60 min and the resulting pellet was used for the kinetic studies. The K_m was determined using an Eadie–Hofstee plot. The pH of the assay medium for all the bicarbonate concentrations examined was in the range 7.9–8.1.

Protein analysis

Protein concentration was determined by the Coomassie Blue method of Bradford (1976), utilizing bovine serum albumin (Sigma) as a standard. When Triton was present in the protein samples, it was in the standards and blanks as well.

RESULTS

ATPase activation and inhibition

Comparisons of ATPase activity resulting from assays performed with a variety of ionic media are presented in Table 1. Bicarbonate significantly increased ATPase activity when compared to activation by $MgSO_4$ alone. The bicarbonate activation can apparently be completely abolished by thiocyanate. Other anions were significantly less effective in stimulating ATPase activity. However, sulfate activation is evident in that $MgSO_4$ activity can be reduced by thiocyanate.

Table 1. Anion and cation effects on the ATPase activity in whole homogenate preparations of Anodonta mantle tissue

Test solution*	Activity	Test solution*	Activity
(N = 3)		(N = 2)	
$MgSO_4$	10.7 ± 3.0	$MgSO_4$	8.5 ± 3.5
$NaHCO_3$	26.7 ± 8.0	$CaSO_4$	8.0 ± 3.5
$NaHCO_3$ + SCN^-	5.3 ± 1.8		
		$NaHCO_3$	35.5 ± 2.5
$NaHCO_3$	35.7 ± 4.3	$NaHCO_3$ + $CaSO_4$	27.0 ± 2.5
$NaHCO_3$ + SCN^-	6.0 ± 2.5		
$NaHCO_3$ + ouabain	35.0 ± 3.1		
NaCl	15.0 ± 2.3	$MgSO_4$	8.5 ± 3.5
NaCl + SCN^-	4.3 ± 2.3	NaCl + KCl	8.0 ± 3.0
NaCl + ouabain	14.0 ± 4.3	NaCl + KCl + SCN^-	4.0 (n = 1)
Na_2SO_4	14.0 ± 4.04	NaCl + KCl + ouabain	6.0 ± 2.0
Na_2SO_4 + SCN^-	5.0 ± 1.2		
Na_2SO_4 + ouabain	18.0 ± 4.0		
$MgSO_4$	10.7 ± 3.0		
$MgSO_4$ + SCN^-	2.5 ± 0.8		

Values are presented as the mean ± standard error of the total enzyme activity (μmol Pi mg^{-1} hr^{-1}).

* All solutions contained 6 mM $MgSO_4$. The concentrations of all other compounds are given in Materials and Methods.

Calcium (1 mM) added to the $MgSO_4$ medium resulted in no activation. However, calcium may slightly inhibit HCO_3^--ATPase activity.

Sodium and potassium, added as the chloride salts, demonstrated no activation, indicating no detectable Na^+,K^+-ATPase. Ouabain (10 mM) tested with all combinations of ions had no effect on any enzyme activity, again indicating no measurable Na^+,K^+-ATPase.

HCO_3^--ATPase

Kinetic studies (Fig. 1) on the modified microsomal fraction resulted in a K_m for HCO_3^- stimulation of 6.83 ± 0.36 mM (mean ± standard error, N = 3). Inhibition of the HCO_3^--ATPase occurred at HCO_3^- concentrations greater than 20 mM.

HCO_3^--ATPase distribution and localization

Following differential centrifugation, HCO_3^--ATPase was distributed most nearly like cytochrome oxidase (Table 2) which suggests a possible mitochondrial location for the ATPase. Altering the centrifugation procedure to obtain a pellet with a very high proportion of cytochrome oxidase activity (Fig. 2)

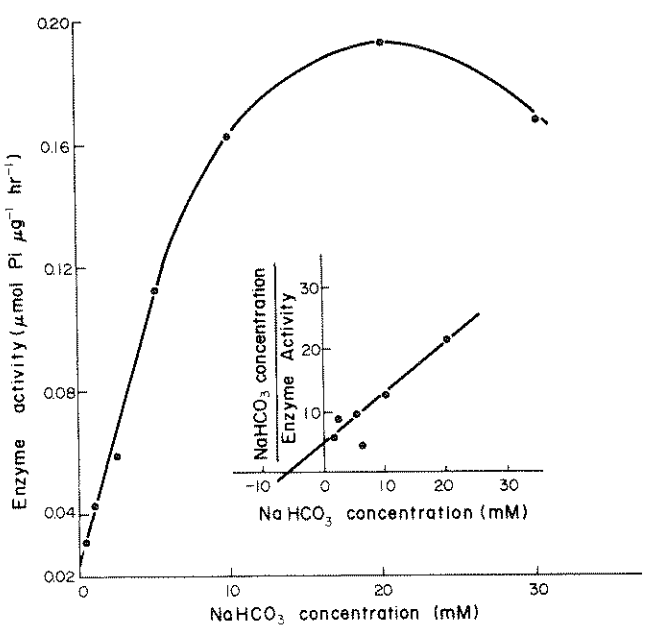

Fig. 1. The effect of HCO_3^- concentration on the HCO_3^--stimulated ATPase activity in a modified mitochondrial fraction obtained by differential centrifugation of *Anodonta* mantle tissue homogenate. The K_m was determined to be 6.83 mM $NaHCO_3$ and the V_{max} approximately 0.025 μmol P_i μg^{-1} hr^{-1} on an Eadie–Hofstee plot (inset). Enzyme activity of axes is represented ×10.

Table 2. Distribution of total enzyme activity after differential centrifugation

Fraction	HCO_3^--ATPase	Cyto. Ox.	Alk. Phosph.	Acid Phosph.
Nuclear (900 × *g*, 10 min)	38.0 ± 9.2	45.8 ± 7.5	30.1 ± 7.9	19.7 ± 6.7
Mitochondrial (14,000 × *g*, 20 min)	33.8 ± 3.4	25.7 ± 4.3	17.8 ± 4.5	24.7 ± 3.6
Microsomal (100,000 × *g*, 60 min)	25.3 ± 7.6	27.9 ± 4.3	42.7 ± 9.7	32.0 ± 5.7
Supernatant	3.1 ± 1.4	0.0	9.2 ± 2.6	19.7 ± 2.5

Values are expressed as the mean ± standard error of per cent enzyme activity recovered ($N = 5$).

produced similar results. However, the HCO_3^--ATPase did not align exclusively with any of the other marker enzymes. In this case, the HCO_3^--ATPase appeared in all fractions, including the final supernatant, a fraction in whcih the cytochrome oxidase activity was not detected, and the heavy mitochondrial fraction, in which acid phosphatase was not detected. These findings rule out a localization for HCO_3^--ATPase exclusively in the lysosomes or mitochondria, possibly implicating the plasma membranes as a location for some of the enzyme.

After isopycnic zonal centrifugation of the whole homogenate (Fig. 3), the distribution of the HCO_3^--ATPase is comparable to that of cytochrome oxidase; that is, the majority of the activity was associated with the denser particles. At the same time, most of the phosphatase activity appeared at lower densities. Again, this supports the idea that a portion of the enzyme is probably located in the mitochondria. However, when a pellet containing most of the cytochrome oxidase activity was recentrifuged using a rate zonal method (Fig. 4), much of the HCO_3^--ATPase activity was separable from that of the cytochrome oxidase and provides additional evidence for a non-mitochondrial location for part of the HCO_3^--ATPase.

DISCUSSION

Enzyme localization

We have reported evidence for both a mitochondrial and non-mitochondrial HCO_3^--ATPase in freshwater clam mantle epithelium using differential rate sedimentation and rate and isopycnic zonal centrifugation. The clam mantle HCO_3^--ATPase cannot be localized to only mitochondria, as suggested for several systems (Table 3), because some of the particulate enzyme activity is completely separable from that of the mitochondrial enzyme, cytochrome oxidase. The multiple locations suggested in this paper agrees with studies on other invertebrate and vertebrate systems (Table 3). There have been some reports of mitochondrial fragments contaminating the microsomal

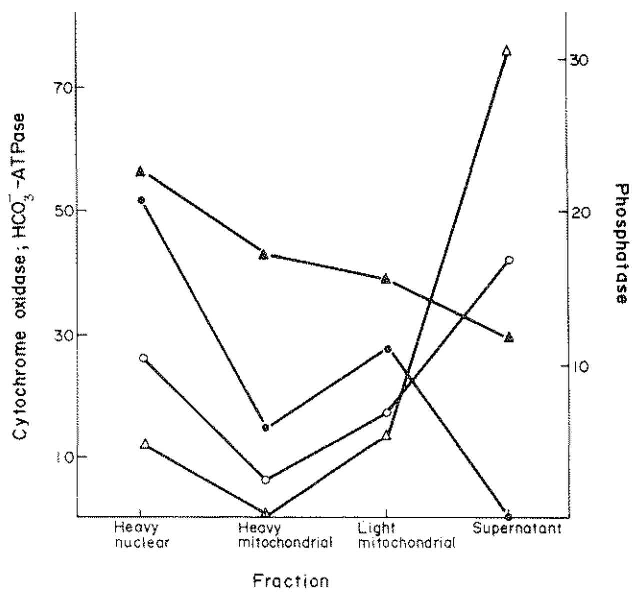

Fig. 2. Distribution of HCO_3^--ATPase and marker enzyme activity in sub-cellular fractions after differential centrifugation. Cytochrome oxidase (●——●); acid phosphatase (△——△); alkaline phosphatase (○——○); and HCO_3^--ATPase (▲——▲). Units for enzyme activity are described in Materials and Methods.

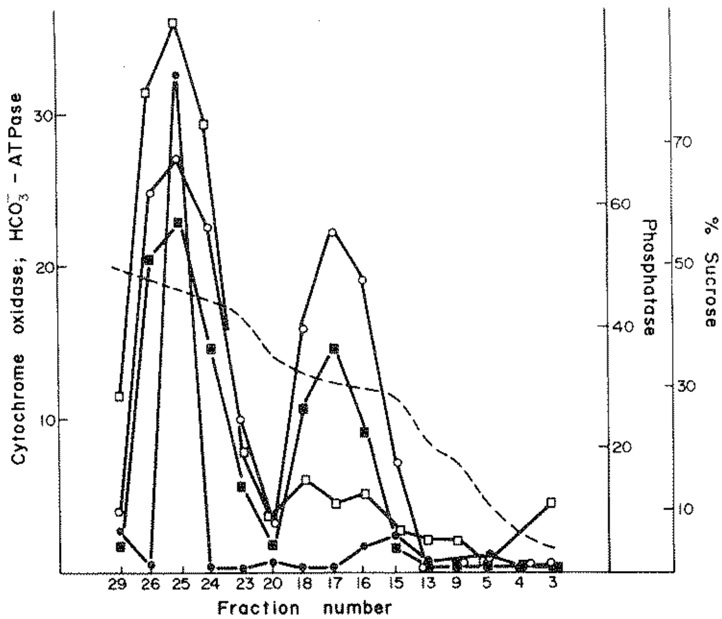

Fig. 3. The distribution of HCO_3^--ATPase and marker enzymes after isopycnic zonal centrifugation of a 105,000 × *g* fraction of whole mantle homogenate. Cytochrome oxidase (□——□); acid phosphatase (■——■); alkaline phosphatase (○——○); and HCO_3^--ATPase (●——●). Sucrose (---) is shown as per cent (w:w). Units of enzyme activity are described in Materials and Methods.

fraction following extremely disruptive tissue homogenization (Kerstetter & Kirshner, 1974; Van Amelsvoort *et al.*, 1977a, b). This may produce enzyme activity in that fraction which would be mistaken for a non-mitochondrial HCO_3^--ATPase. In the case reported here, the separability of the enzyme from the mitochondrial marker suggests its non-mitochondrial location. However, outer mitochondrial membranes could be removed during homogenization and might be separable from the inner membranes and thus cytochrome oxidase.

Because there was HCO_3^--ATPase activity that could not be completely separated from alkaline phosphatase by the techniques employed in this study, some of the non-mitochondrial ATPase may be plasma membrane bound, also suggested by several workers (Table 3). Related to this, Neff (1972) found that most outer mantle epithelial cells of the marine

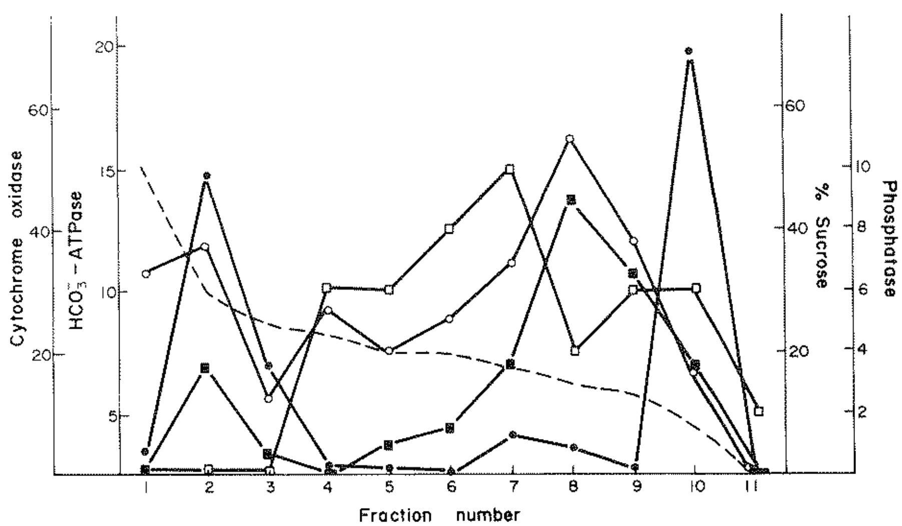

Fig. 4. Distribution of HCO_3^--ATPase and marker enzymes in subfractions obtained by rate zonal centrifugation of a mitochondrial fraction. Cytochrome oxidase (□——□); acid phosphatase (■——■); alkaline phosphatase (○——○); and HCO_3^--ATPase (●——●). Sucrose (---) is shown as per cent (w:w).

Table 3. Literature survey: Subcellular localizations for HCO_3^--ATPase activity

Tissue	Reference	Fraction M[1]	N–M[2]	PM[3]	L[4]
Gastric mucosa	Blum *et al.* (1971)	×		×	
	Narumi & Kanno (1973)	×		×	
	Sachs *et al.* (1972)	×	×		
	Soumarmon *et al.* (1974)	×		×	
	Spenney *et al.* (1973)	×		×	
	Van Amelsvoort *et al.* (1977a)	×			
Rumen forestomach	Hegner & Anika (1975)	×*		×	
Pancreas	Simon *et al.* (1972a)	×	×		
	Simon & Thomas (1972)		×		
Submandibular gland	Simon *et al.* (1972b)	×	×		
	Itzusu & Siegel (1972)	×			
Uterus	Iritani & Wells (1976)	×		×	×*
Liver	Grisola & Mendelson (1974)	×			
	Iritani & Wells (1974)				×
	Itzusu & Siegel (1975)	×			
Brain	Kimelberg & Bourke (1973)	×			
Kidney	Cole (1978)	×	×		
	Kinne-Saffron & Kinne (1974)		×		
	Liang & Sacktor (1976)	×		×	
	Van Amelsvoort *et al.* (1977b)	×			
Frog liver	Grisola & Mendelson (1974)	×			
Teleost gill	DeRenzis & Bornancin (1977)	×		×	
	Kerstetter & Kirschner (1973)	×**		×**	
	Van Amelsvoort *et al.* (1977a)	×			
	Ho & Chan (1981)	×			
Necturus oxyntic cells	Wiebelhaus *et al.* (1971)	×		×	
Fiddler crab gill	DePew & Towle (1979)	×		×	
Oyster mantle epithelium	Wheeler (1975)	×		×	

[1]Mitochondrial; [2]Non-mitochondrial; [3]Plasma membrane/microsomal; [4]lysosomal
*Possible location; **Preliminary data.

clam *Mercenaria mercenaria* have few internal membranes, ruling out intracellular membranes as a major location for a HCO_3^--ATPase. However, a lysosomal location for HCO_3^--ATPase has been described (Iritani & Wells, 1974, 1976) and cannot be completed discounted because some ATPase activity was not entirely separable from acid phosphatase using the techniques employed here. On the other hand, there is HCO_3^--ATPase activity that is definitely separable from acid phosphatase, ruling out an exclusively lysosomal location for the ATPase.

Anion and cation effects

The failure to find significant Cl^- activation in the absence of HCO_3^-, reported in this study, has also been reported for the goldfish gill (DeRenzis & Bornancin, 1976). However, Cl^- has been reported to stimulate ATPase in the fiddler crab gill (DePew & Towle, 1979) and inhibit $MgSO_4$ stimulated activity in rat liver (Itzutsu & Siegel, 1975).

In the clam mantle system, SO_4^{2-} activation occurred in the absence of HCO_3^- as evidenced by the sensitivity of $MgSO_4$-activated ATPase to thiocyanate. Sulfate also stimulated the ATPase in the goldfish gill, but only in the presence of HCO_3^- (DeRenzis & Bornancin, 1976). However, the anion is ineffective in two mammalian systems, the liver (Iritani & Wells, 1974) and kidney (Liang & Sacktor, 1976). These differences in SO_4^{2-} activation may indicate a difference in anion specificity in mammalian and non-mammalian systems.

There is little specific precedence for the Ca^{2+} inhibition of HCO_3^--stimulated ATPase reported here. However, 5 mM Ca^+ inhibited HCO_3^--ATPase activity for the frog and rat liver mitochondria (Grisola & Mendelson, 1974). Contrary evidence has been provided for rat uterus (Iritani & Wells, 1976) in which a Ca^{2+}-ATPase was stimulated further by the addition of 20 mM HCO_3^-. No explanation can be offered for the differences in sensitivity of HCO_3^--ATPase to Ca^{2+} in these various systems.

Function of HCO_3^--ATPase in mantle tissue

Several functions have been proposed for HCO_3^--ATPase of other secretory tissues. Usually, these functions are attributed to a plasma membrane-bound enzyme. These include acid secretion by the gastric mucosa (Blum, 1971; Narumi & Kanno, 1973; Sachs *et al.*, 1972), HCO_3^- reabsorbtion in the kidney (Cole, 1979; Kinne-Saffran & Kinne, 1974; Liang & Sacktor, 1976) and the movement of Cl^- across the gills of teleost fish (DeRenzis & Barnancin, 1977; Kerstetter & Kirschner, 1974) and fiddler crab (Depew & Towle, 1979). Thiocyanate, a known inhibitor of HCO_3^--ATPase also inhibits acid secretion in the gastric mucosa (Blum, 1971) and has an effect on HCO_3^-/Cl^- exchanges in the goldfish gill (DeRenzis, 1975).

Shell mineralization is primarily the function of the epithelia of mantle tissue. Because HCO_3^- is the major form of dissolved inorganic carbon in blood or media, a plasma membrane-bound transport system

Table 4. Literature suvery: Kinetic data for HCO_3^--stimulated ATPase.

Tissue	Reference	K_m (mM HCO_3^-)	V_{max} (μmol Pi mg^{-1} hr^{-1})	Max. Stim.[1] (mM HCO_3^-)	Inhibition[2] (mM HCO_3^-)
Gastric mucosa	Van Amelsvoort *et al.* (1977a)				>25*; >50*
	Blum *et al.* (1971)	2.65 ± 0.04	46.47		
	Soumarmon *et al.* (1974)			80	
Pancreas	Simon & Thomas (1972)			20	
Submandibular gland	Izutsu & Siegel (1972)	2.0			
Uterus	Iritani & Wells (1976)	3.29*			
		3.34*			
Liver (lysosomal)	Iritani & Wells (1974)	7.25	0.83	30	30
Liver	Izutsu & Siegel (1975)	6.0		25	25
Brain	Kimelberg & Bourke (1973)			20–30	
Kidney	Cole (1979)		8.2 ± 0.9	50	
	Kinne-Saffron & Kinne (1974)	16.0		50	
	Liang & Sacktor (1976)	0.37	0.50		
Rumen forestomach	Hegner & Anika (1975)			25	
Trout gill	Kerstetter & Kirschner (1974)	16.0			
Eel gill	Ho & Chan (1981)				
	(freshwater)	6.26 ± 0.92	15.1 ± 1.3	30–40	50
	(saltwater)	4.01 ± 0.66	19.0 ± 1.86		
Clam mantle	Present study	6.83	25.5	20	20

[1] HCO_3^- concentration giving maximum stimulation; [2] HCO_3^- concentration above which inhibiton occurred.
* Subfractions.

for this ion could facilitate supply of carbonate for the mineralizing shell. In support of this, a bicarbonate ATPase has been described in the American oyster, and it has been suggested that this enzyme is membrane-bound (Wheeler, 1975). In addition, Wheeler (1975) also has found evidence for an active HCO_3^- transport mechanism in oyster mantle epithelium.

An active control mechanism to regulate Cl^- balance between the blood and external media of freshwater clams has been described by Dietz & Branton (1979). This mechanism is thought to be Cl^-/base exchange and HCO_3^- has been suggested as the exchange base. It is also interesting to note that the Cl^- exchange is inhibited by thiocyanate. In addition, an active Cl^-/HCO_3^- exchange has been demonstrated in the gill tissues of goldfish (DeRenzis, 1975; DeRenzis & Bornancin, 1977), toadfish (Kormanik & Evans, 1979), and trout (Kerstetter & Kirschner, 1974). These tissues are also known to contain HCO_3^--ATPase and it has been proposed that this enzyme functions in ionic regulation. In light of the fact that other anions (SO_4^{2-}) may stimulate ATPase and because one surface of the rather extensive mantle tissue separates the blood compartment from the medium, the HCO_3^--ATPase of this tissue could conceivably be involved in ion regulation by exchange processses similar to those proposed for gill tissues. The possible involvement of mantle in anion regulation has also been suggested by Dietz and Branton (1979).

Kinetics

Kinetic data for HCO_3^--ATPase from various tissues is presented in Table 4. Most of the data is from mammalian systems; however, the data presented in this study is in general agreement with the findings of other workers. Despite the similarities, differences do exist. In all, no clear trends emerge from studying the comparative data.

Clams collected in the winter show specific activities and K_m's for HCO_3^--ATPase which are lower than for the clams used in the present study, which were collected in the summer and early fall (Wheeler, 1981). This may suggest the presence of more than one isozyme of HCO_3^--ATPase in the clam mantle epithelium, which is not surprising in light of the location of the enzyme in more than one subcellular fraction.

The inhibition of HCO_3^--ATPase at concentrations of $NaHCO_3$ greater than 20 mM may be due to increasing the ionic strength of the assay media as suggested by Van Amelsvoort *et al.* (1977a). This hypothesis is in agreement with the results of one experiment (not presented) in which the ATPase activity due to 10 mM $NaHCO_3$ was reduced by 60% when 30 mM sodium and 15 mM potassium sulfate were added to the assay medium.

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