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Mitochondrial carbonic anhydrase

($C^{16}O^{18}O$ exchange/HCO_3^- permeability/acetazolamide/carbonate dehydratase)

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ABSTRACT **We have assayed carbonic anhydrase activity (carbonate dehydratase, carbonate hydro-lyase, EC 4.2.1.1) and bicarbonate permeability in suspensions of broken and intact guinea pig mitochondria by monitoring the disappearance of $C^{16}O^{18}O$. We found significant activity in preparations from liver and skeletal muscle, but not in preparations from heart muscle, brain, and kidney. Intact mitochondria containing carbonic anhydrase produce a two-phase acceleration of the disappearance of the labeled CO_2, which indicates that the enzyme is located in a region more accessible to CO_2 than to HCO_3^-. Acetazolamide inhibits the enzyme activity instantly in broken mitochondria but only after a delay in intact mitochondria, indicating that the enzyme is in a region not immediately accessible to the inhibitor. Sonication of mitochondria containing carbonic anhydrase activity releases the enzyme, which remains in the supernatant after sedimentation of the submitochondrial particles. This shows that mitochondrial carbonic anhydrase is in the matrix compartment and not in, or bound to, the inner membrane. The activity of the enzyme increases markedly with increasing pH. The enzyme activity of intact mitochondria is greater than that of the broken mitochondria at the same pH of the suspending fluid, corresponding to an intramitochondrial pH that is 0.2–0.5 unit more alkaline.**

CO_2 plays an important role in mitochondrial metabolism. The enzymes of the tricarboxylic acid cycle that produce CO_2 (1) are located within the mitochondrial matrix (2), as are those enzymes that fix CO_2 in the pathways of gluconeogenesis and urea production (3, 4). Prior to 1972, the few studies of the interaction of CO_2 and HCO_3^- with mitochondria dealt with the permeability of the inner mitochondrial membrane to these two species and with the possible existence of a mitochondrial carbonic anhydrase. Chappell and Crofts (5) showed that the inner mitochondrial membrane was essentially impermeable to HCO_3^- but readily permeable to CO_2, a finding now widely accepted (6). Early reports of mitochondrial carbonic anhydrase (7–9) were cautious because of the problem of contamination, with the exception of that of Karler and Woodbury (10). Rossi (11) found 4% of the carbonic anhydrase of rat liver homogenate in the mitochondrial fraction and showed that it was an intramitochondrial enzyme. Mitochondria incubated with 10 μM acetazolamide showed no activity. Holton (12) found carbonic anhydrase activity in isolated rat liver mitochondria with both penetrant CO_2 and nonpenetrant HCO_3^- as substrates. In contrast to these two positive findings with liver mitochondria, Deprez and Francois (13) found no carbonic anhydrase activity in mitochondrial preparations from various tissues, including liver. In all the above studies, carbonic anhydrase activity was measured by change in pH.

A functional role for CO_2/HCO_3^- in providing a counteranion for energy-linked mitochondrial Ca^{2+} uptake was proposed by Elder (14). Elder and Lehninger (15, 16) showed that HCO_3^- as such cannot serve as counteranion for Ca^{2+} in this uptake by rat liver mitochondria because it is impermeant in the mitochondrial membrane, but that CO_2 could provide an intramitochondrial counteranion and so facilitate energy-linked Ca^{2+} accumulation as $CaCO_3$ in the matrix. This facilitation of Ca^{2+} uptake was sensitive to inhibition by acetazolamide, unlike that provided by another important intracellular anion, inorganic phosphate (17). The acceleration of O_2 uptake by CO_2 in rat liver mitochondria was also observed by Harris (18, 19) under conditions where the movements of endogenous mitochondrial inorganic phosphate between mitochondrial matrix and suspending medium (20) were controlled. Harris (19) further observed that CO_2 did not affect energy-linked Ca^{2+} uptake in rat heart mitochondria, suggesting that the presence of intramitochondrial carbonic anhydrase may be tissue specific.

The development of a technique for assaying carbonic anhydrase (carbonate dehydratase, carbonate hydro-lyase, EC 4.2.1.1) in intact erythrocytes (21, 22) by means of isotopic exchange of $C^{16}O^{18}O$ provided a means for re-examining the sometimes conflicting, earlier studies of mitochondrial carbonic anhydrase.

MATERIALS AND METHODS

Isolation of Intact Mitochondria. Guinea pigs fasted overnight and were killed by decapitation. The tissue from which mitochondria were to be prepared was excised as rapidly as possible and the mitochondria were isolated. Mitochondrial oxidative activity was assayed by the method of Estabrook (23); mitochondrial protein was determined by the method of Miller (24).

Liver. Mitochondria were isolated in 225 mM mannitol/75 mM sucrose/1.0 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetate (EGTA) at 250 mosM, pH 7.0, ionic strength $\approx$ 0 (solution I) as described (25). They were washed once in 225 mM mannitol/75 mM sucrose (solution II) and once in solution II containing 25 mM $NaHCO_3$ at pH 7.4 (solution III) and then resuspended in solution III to a final concentration of 80–100 mg of protein per ml.

Kidney. The procedure was the same as for liver mitochondria.

Heart. The procedure of Mela and Seitz (25) with Nagarse (Enzyme Products, New York) was used. The recovery and washing procedure was similar to that for liver mitochondria.

Skeletal muscle. Mitochondria were prepared from skeletal muscle (mixed types) excised from the hind legs of two guinea pigs, exactly following the procedure for rabbit leg muscle described by Storey *et al.* (26). The final mitochondrial suspension contained 30–50 mg of protein per ml of solution III.

Other Mitochondrial Preparations. Liver submitochondrial particles were prepared by sonication in 20 mM Tris·HCl (pH 7.4) with a Branson sonifier at 50 W for 30 sec. The sonicated suspension was centrifuged at 11,000 × *g* for 15 min; the sub-

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Abbreviations: solution I, 225 mM mannitol/75 mM sucrose/1.0 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetate, pH 7.0; solution II, 225 mM mannitol/75 mM sucrose; solution III, solution II containing 25 mM $NaHCO_3$ at pH 7.4.

mitochondrial particles were recovered by centrifugation of the resulting supernatant at 107,000 × *g* for 60 min to separate the submitochondrial particles from the matrix fraction. These were resuspended in a minimal volume of solution III. "Broken" liver mitochondria were prepared either by freezing overnight and then thawing or by addition of sodium cholate to 1% final concentration. "Broken" skeletal muscle mitochondria were prepared in 1% cholate.

Lysosomes. These were sedimented at 13,000 × *g* for 20 min from the supernatant of solution I obtained after isolation of liver and kidney mitochondria by centrifugation at 7700 × *g* for 14 min.

Determination of Carbonic Anhydrase Activity. *Apparatus.* The glass chamber for the continuous measurement of $C^{16}O^{16}O$ and $C^{16}O^{18}O$ has been described (21). The ion source of a mass spectrometer (Consolidated Electronics, Pasadena, CA; model 21-620 A) was separated from the reaction fluid by a thin Teflon membrane supported by a sintered glass disc. The pH was constantly monitored by a combination glass pH electrode (Radiometer model GK 2641 C). The temperature of the chamber was regulated by circulating water through a jacket. The response time of the entire system to a change of CO_2 partial pressure in the reaction mixture, including mixing time and mass spectrometer memory for $C^{16}O^{18}O$, is 3 sec.

Carbonic anhydrase assay. Solutions were prepared immediately before each assay by adding solid ^{18}O-enriched sodium bicarbonate (25 mM), prepared by exchange reaction of unlabeled bicarbonate and ^{18}O-labeled water, to either water or solution II. The former solution was used for assays with broken mitochondria, the latter for assays with intact mitochondria. The reaction solution was introduced rapidly into the reaction vessel and the pH was adjusted to the desired value by adding either 0.5 M H_2SO_4 or 1 M NaOH with a microsyringe. The peaks of mass 44 ($C^{16}O^{16}O$) and mass 46 ($C^{16}O^{18}O$) were monitored alternately every 30 sec throughout the assay. The assay was continued for approximately 1 hr after addition of between 15 and 200 μl of the suspension and was considered to be complete when the mass 46 peak remained constant for 5 min.

Calculation of carbonic anhydrase activity. The mass 46 peak, $C^{16}O^{18}O$, decreases as ^{18}O exchanges with the 55 M ^{16}O pool in water according to the schema of Mills and Urey (27):

$$\begin{array}{l} C^{16}O^{18}O \\ \quad + \\ H_2{}^{16}O \underset{{}^{2}/_{3}k_{H_2CO_3}}{\overset{k_{CO_2}}{\rightleftharpoons}} H_2C^{18}O^{16}O_2 \rightleftharpoons HC^{18}O^{16}O_2^- + H^+ \\ \qquad\qquad\qquad\qquad \Big\downarrow {}^{1}/_{3}k_{H_2CO_3} \\ \qquad\qquad\qquad\qquad C^{16}O_2 + H_2{}^{18}O \end{array} \quad [1]$$

Itada and Forster (21) developed a theoretical model to describe mass 46 disappearance in erythrocyte suspensions. It includes assumptions of intra- and extracellular phases in which exchange reactions take place but at different rates, instantaneous equilibration of CO_2 between the phases, and flux of bicarbonate proportional to its concentration difference across the membrane. By measuring the exponential constant of the more slowly decreasing term and the relative magnitude of the more rapidly decreasing term from the biphasic experimental disappearance curve of mass 46, we can calculate carbonic anhydrase activity inside the cell (k_{cat} in sec^{-1}) and the exchange permeability of the cell wall to HCO_3^- (P in cm sec^{-1}) by means of a computer program developed by Martin Pring.

Mitochondrial surface area is needed to calculate exchange permeability, which fortunately is so low that HCO_3^- exchange is of limited significance. Mitochondrial water volume is needed to calculate k_{cat} in the matrix, but lacking this datum we have instead calculated k_{cat} in ml sec^{-1} mg^{-1} divided by mitochondrial protein concentration, std k_{cat}. In terms of enzyme kinetics (28),

$$\text{std } k_{cat} = \frac{V_{max}}{[CO_2]\left(1 + \frac{k_{-2}}{k_{-1}}\right) + K_m} \times \frac{1}{[\text{mitochondrial protein}]} \quad [2]$$

in which V_{max} and k_m have the classical Michaelis–Menten kinetics definition for the hydration reaction; k_{-1} is the reaction velocity constant in sec^{-1} for the dissociation of the enzyme–substrate complex to form CO_2; and k_{-2} is the reaction velocity constant in sec^{-1} for the dissociation of the enzyme–substrate complex to form H_2CO_3 or HCO_3^-.

In the lysate we can use the equation of Mills and Urey (27) to obtain an effective reaction velocity constant equal to $k_{cat} + k_{CO_2}$, from which we subtract k_{CO_2} to obtain k_{cat}.

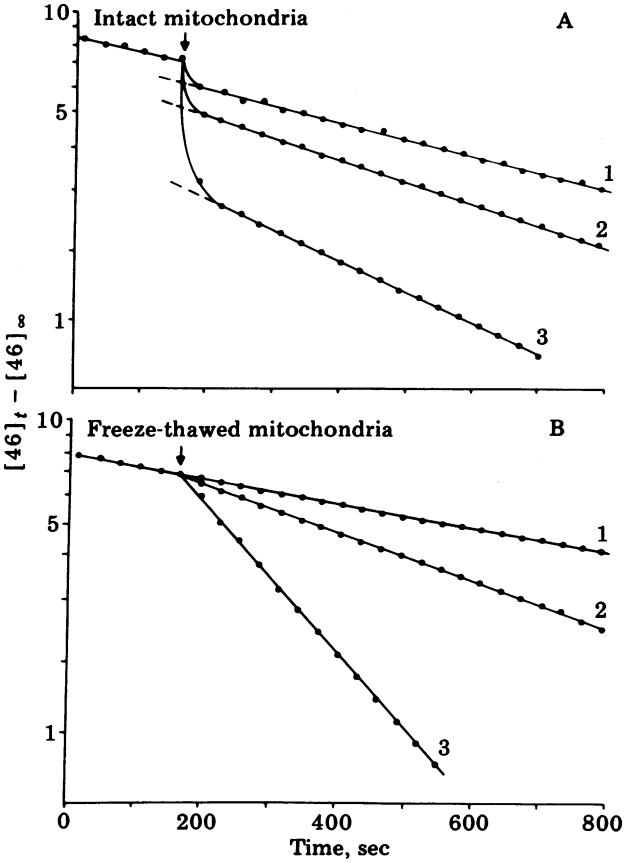

FIG. 1. Plots of $\log_{10}$(mass 46 peak height at time t, $[46]_t$, minus mass 46 peak height at equilibrium, $[46]_\infty$) against time before and after guinea pig liver mitochondria were added in 25 mM $NaHCO_3$ 2% enriched with $NaHC^{16}O_2{}^{18}O$ in 300 mosM mannitol/sucrose (solution III) at 25°C and pH 7.4. (*A*) Effect of increasing the concentration of mitochondria at pH 7.40. A sufficient volume of suspension was added (arrow) to bring the protein concentration to 0.15 (curve 1), 0.40 (curve 2), and 0.88 (curve 3) mg/ml. (*B*) Freeze-thawed mitochondria were added (arrow) to produce a final protein concentration of 0.28 (curve 2) and 1.88 (curve 3) mg/ml in the reaction mixture which had been adjusted to pH 7.4. In comparable experiments with inhibitor, 10 μM acetazolamide was added to the solution before the pH was adjusted (curve 1).

RESULTS

In Fig. 1*A* are shown graphs of $\log_{10}$(mass 46 peak height − mass 46 peak height at final equilibrium) against time in 25 mM labeled $NaHCO_3$ in mannitol/sucrose (solution III) at pH 7.4 and 25°C in the presence of intact liver mitochondria. Before addition of the mitochondria, the mass 46 peak decreased exponentially as the uncatalyzed reactions of CO_2 and H_2CO_3 caused the exchange of ^{18}O with the large pool of ^{16}O in water. Upon addition of mitochondria, the mass 46 peak decreased rapidly with a half-time of about 10 sec. This exponential phase was followed by a second slower exponential process. The height of the mass 46 peak at the time of addition of the mitochondria divided by the height of this peak in the second slow exponential phase extrapolated back to the arrow is called the step ratio. As the concentration of mitochondria in the suspension increased, both the step ratio and the negative slope of the second exponential phase increased. In Fig. 1*B* is shown the acceleration of mass 46 disappearance caused by broken mitochondria without the step seen with intact mitochondria. The presence of 10 μM acetazolamide, a potent and specific inhibitor of carbonic anhydrases, completely inhibits activity.

The experiments in Fig. 2*A* were carried out by adding intact liver mitochondria at similar final protein concentrations to solution III that had been adjusted to pH 7.00, 7.40, or 7.80. The lower the pH, the greater was the negative slope of the uncatalyzed disappearance of the mass 46 peak (21, 27), the smaller was the step ratio, and the greater was the negative slope of the second phase after addition of mitochondria. If the inner mitochondrial membrane was first damaged by either freezing and thawing or cholate treatment, the plots shown in Fig. 2*B* were obtained. There was no step, and the lower the pH, the greater was the negative slope seen after addition of the broken mitochondria. These results are parallel to those seen with lysed, as compared with intact, erythrocytes (21); they strongly imply that liver mitochondria have carbonic anhydrase activity in the matrix that is readily accessible to CO_2, but not to HCO_3^-, in the intact state.

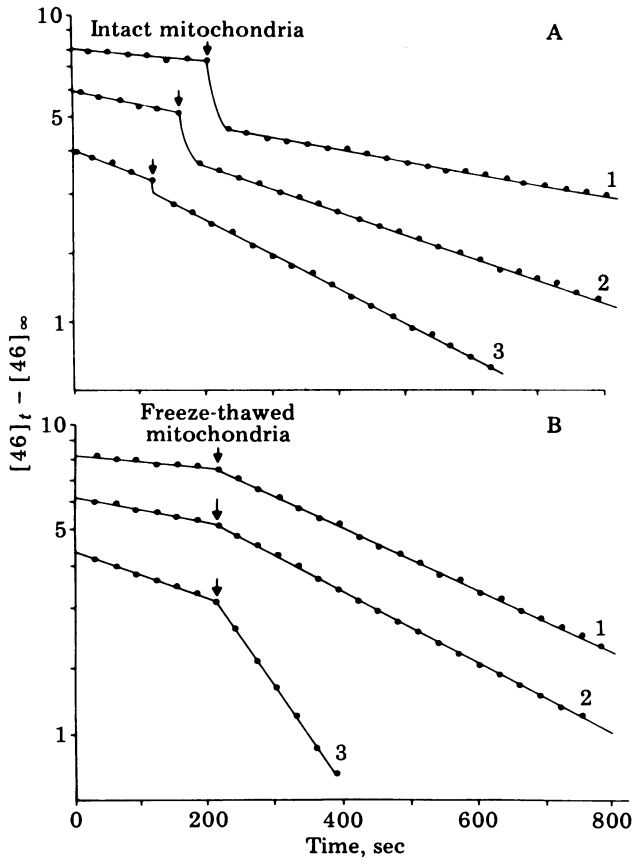

FIG. 2. Semilogarithmic plots as in Fig. 1 for intact (*A*) and broken (*B*) guinea pig liver mitochondria at three different pH values of the suspending medium. (*A*) The mitochondrial suspension was added (arrow) to bring the protein concentration of the mixture to: curve 1, 0.42 mg/ml (pH 7.80); curve 2, 0.39 mg/ml (pH 7.40); curve 3, 0.39 mg/ml (pH 7.00). (*B*) The freeze-thawed mitochondrial suspension was added (arrow) to produce 0.55 mg of protein per ml in reaction solutions that had been adjusted to pH values of 7.75 (curve 1), 7.40 (curve 2), and 7.00 (curve 3). Other conditions were the same as for Fig. 1.

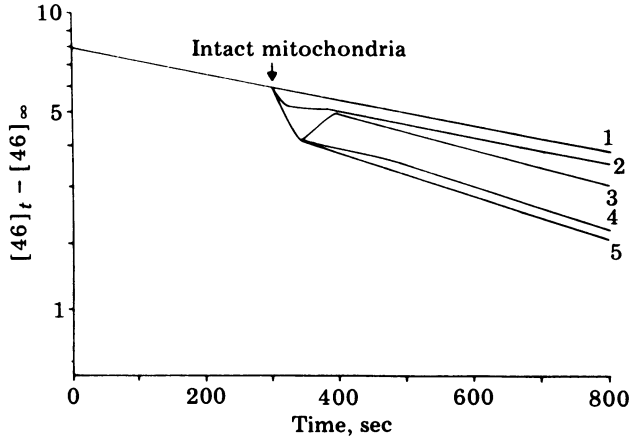

FIG. 3. Semilogarithmic plot as in Figs. 1 and 2 for intact mitochondria (0.52 mg of protein per ml) at pH 7.40. Acetazolamide was added to the reaction solution before addition of mitochondria: curve 1, 100 μM; curve 2, 5 μM; curve 3, 1 μM; curve 4, 0.25 μM; curve 5, 0 μM. The dots indicating peak heights have been left off the diagram for clarity.

The experiments presented in Fig. 3 show the effect of four different concentrations of acetazolamide in solution III before addition of intact mitochondria. In the presence of 0.25 μM (curve 4) and 1 μM (curve 3) acetazolamide, the trace for the mass 46 peak yielded the expected step ratio, but it then rose, achieving a new disappearance rate in 1–3 min. With 5 μM acetazolamide (curve 2), the inhibition occurred before the mass 46 peak could fall only part way to the control level. With 100 μM (curve 1), the inhibition occurred so rapidly that the mass 46 peak did not fall below the uncatalyzed rate. These results

Table 1. Carbonic anhydrase activity in freeze-thawed guinea pig mitochondria at 25°C and pH 7.40

Tissue	No. of experiments*	Specific activity,† μmol CO_2 converted mg^{-1} min^{-1}	Std k_{cat},‡ sec^{-1} mg^{-1} ml
Kidney	4 (3)	0§	0
Liver	8 (4)	11.5 ± 2.0	0.130 ± 0.024
Heart	2 (2)	0§	0
Brain	2 (2)	0§	0
Skeletal muscle	3 (3)	5.9 ± 0.4	0.067 ± 0.005

Values are expressed as mean ± SD.

* Number of mitochondrial preparations is in parentheses.

† Specific activity in μmol of CO_2 per min per mg of protein per ml = $k_{cat}[CO_2]$ in mM × 60. At 25°C and pH 7.40, the concentration of CO_2 is 1.47 mM. Note that std k_{cat} is the equivalent reaction velocity constant for the enzyme-catalyzed hydration of CO_2 and equals k_{cat} divided by the concentration of mitochondrial protein in mg/ml. Std k_{cat} can also be described in terms of Michaelis–Menten kinetics by Eq. 2. Because K_m, k_{-1}, and k_{-2} are not known, V_{max} cannot be calculated.

‡ Mitochondrial protein concentration ranged from 1 to 14.5 mg/ml. There was no significant variation in std k_{cat} with mitochondrial protein concentration.

§ In these experiments there was no increase in the hydration rate over the uncatalyzed rate upon the addition of over 10 mg of mitochondrial protein per ml.

Table 2. Carbonic anhydrase activity in intracellular organelles of guinea pig tissues at pH 7.40, 25°C, and ionic strength 25

Tissue	Intact mitochondria: Mitochondria	Intact mitochondria: Supernatant	Sonicated mitochondria: Submitochondrial particles	Sonicated mitochondria: Matrix fraction*	Lysosomes
Liver					
Std k_{cat} (sec^{-1} mg^{-1} ml)†	0.409 ± 0.061	0	0	0.379 ± 0.230	0
No. of preparations	5	2	2	2	2
No. of experiments	6	2	2	3	2
Protein (mg/ml, range)	1–14.5	—	—	—	1–5
Kidney					
Std k_{cat} (sec^{-1} mg^{-1} ml)†	0	0	—	—	0
No. of preparations	3	2	—	—	2
No. of experiments	4	2	—	—	2
Protein (mg/ml, range)	1–20	—	—	—	1–5

* The supernatant over the intact mitochondria after the final wash was used.

† Std k_{cat} is defined in the legend of Table 1. Mean ± SD is shown.

are again consistent with the existence of carbonic anhydrase in the matrix, which is shielded from the suspending medium by the inner mitochondrial membrane through which acetazolamide diffuses at a rate proportional to its concentration (5, 16, 21).

Calculations of the exchange permeability for HCO_3^- of the mitochondrial inner membrane by the method of Itada and Forster (21) yielded values so low that it could not be determined with precision. It was estimated to lie in the range of 10^{-6}–10^{-5} cm/sec, much less than that of the human erythrocyte, which averages 7×10^{-4} cm/sec (22). This result is in accord with previous reports (5, 16), which placed HCO_3^- in the category of anions impermeant to the inner membrane of liver mitochondria.

Table 1 summarizes the measurements of carbonic anhydrase activity in mitochondria from different tissues of the guinea pig. Liver mitochondria have significant carbonic anhydrase; skeletal muscle mitochondria have about one-third as much. Heart, brain, and kidney mitochondria have no detectable carbonic anhydrase activity.

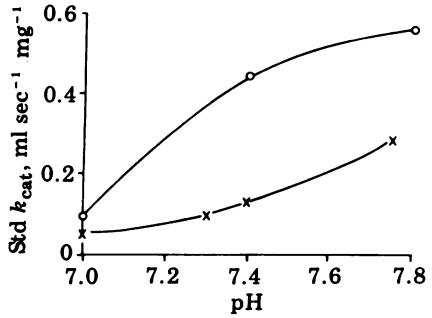

FIG. 4. Plot of std k_{cat} against pH of the reaction mixture for intact (O) and freeze-thawed (X) liver mitochondria. Other conditions were the same as for Fig. 1.

The absence of detectable carbonic anhydrase activity in kidney mitochondria provided a further control with regard to the provenance and location of carbonic anhydrase in liver mitochondria, as shown in Table 2. Neither intact kidney mitochondria nor the supernatant from the final mitochondrial wash contained detectable carbonic anhydrase activity, nor was activity detectable in kidney lysosomes. Rat renal tubular cells contain a high content of carbonic anhydrase, 97% of it cytosolic, with the remainder in the brush border and basal-lateral regions of the plasma membrane (29); we assume the same is true for guinea pig kidney. None of this enzyme was entrained by either mitochondria or lysosomes, showing that these organelles are free of cytoplasmic or plasma membrane carbonic anhydrase. Liver lysosomes also have no detectable carbonic anhydrase; thus liver mitochondrial carbonic anhydrase cannot arise from lysosomal contamination. Sonication of isolated liver mitochondria yielded a soluble fraction containing the enzyme, confirming the conclusion drawn from $C^{16}O^{18}O$ exchange that this enzyme is in the mitochondrial matrix (2).

The $C^{16}O^{18}O$ exchange technique provides a convenient method for estimating the pH of the mitochondrial matrix space. The increase in std k_{cat} with increasing pH is shown for broken mitochondria in Table 3. It was independent of mitochondrial protein concentration in the reaction medium. In intact mitochondria, the activity was always higher than that observed in broken mitochondria at a given pH of the medium, implying that the mitochondrial matrix was more alkaline. This is shown in Fig. 4, in which the carbonic anhydrase activity, expressed as k_{cat}/mg of protein (std k_{cat}), is plotted for intact

Table 3. Variation in guinea pig liver mitochondrial carbonic anhydrase with pH at 25°C

Mitochondria	pH	No. of experiments*	Uncatalyzed reaction (k_{CO_2}),† sec^{-1}	Catalyzed reaction: Std k_{cat}, sec^{-1} mg^{-1} ml	Catalyzed reaction: Specific activity, μmol mg^{-1} min^{-1}
Intact	7.00	6 (3)	0.0357 ± 0.0038	0.093 ± 0.033	19.0 ± 6.7
	7.40	6 (5)	0.0451 ± 0.0042	0.445 ± 0.066	39.2 ± 5.8
	7.80	4 (3)	0.0484 ± 0.0105	0.558 ± 0.096	20.1 ± 3.5
Freeze-thawed	7.00	4 (3)	0.0352 ± 0.0027	0.049 ± 0.007	10.1 ± 6.4
	7.30	1 (1)	0.0448	0.098	10.8
	7.40	8 (4)	0.0458 ± 0.0038	0.130 ± 0.024	11.5 ± 2.0
	7.75	4 (3)	0.0478 ± 0.0033	0.285 ± 0.025	11.7 ± 2.6
Cholate	7.40	2 (2)	0.0459	0.134	11.8

Values are expressed as mean ± SD.

* The number of mitochondrial preparations is in parentheses.

† k_{CO_2} is the reaction velocity constant for the uncatalyzed hydration of CO_2, calculated from the half-time of $C^{16}O^{18}O$ disappearance.

and broken mitochondria as a function of the pH of the suspending medium. A matrix pH that is 0.2–0.5 unit more alkaline is calculated for guinea pig liver mitochondria respiring with endogenous substrate, in agreement with the cytosol/matrix ΔpH value of 0.4 (matrix alkaline) obtained with isolated rat hepatocytes (30).

DISCUSSION

The exchange of ^{18}O from $C^{16}O^{18}O$ into $H_2{}^{16}O$, as measured by mass spectrometry (21), is the only technique of which we are aware that can directly determine carbonic anhydrase activity inside a cell or vesicle. The presence of carbonic anhydrase in a space where it is more available to CO_2 than to HCO_3^- produces a characteristic two-phase exponential disappearance curve for mass 46, the "step ratio," which is clearly diagnostic, even in the presence of significant contaminating carbonic anhydrase in the suspending fluid. Other published methods (10) involve observing the effect on pH of the medium of reactions of CO_2 with cell suspensions and are presumably rate limited by the accumulation of end products or depletion of reactants within the cell. The end product of $C^{16}O^{18}O$ exchange is labeled water, the accumulation of which should not become significant because it is diluted in 55 M unlabeled water in the cell and can exchange easily with unlabeled water outside.

Application of the mass spectrophotometric technique to the determination of carbonic anhydrase activity in mitochondria has shown that liver and skeletal muscle mitochondria have the enzyme, that heart, brain, and kidney mitochondria do not, and that carbonic anhydrase is a matrix enzyme in those mitochondria in which it is found. This result is consistent with earlier reports concerning liver mitochondria (7–11), and provides quantitative comparison between the mitochondria of different tissues. The lack of the enzyme in heart mitochondria explains the observation of Harris (19) that CO_2 had no effect on energy-linked Ca^{2+} uptake in these organelles, in contrast to the stimulation of Ca^{2+} uptake by CO_2 observed with liver mitochondria (15, 16, 18, 19).

The $C^{16}O^{18}O$ exchange technique also provides a means of assessing the pH of the matrix space by direct measurement of this activity in intact mitochondria. The pH value estimated in this study for guinea pig liver mitochondria under the experimental conditions used for the enzyme assay was remarkably close to that measured for the pH in isolated rat hepatocytes (30), indicating that the method is reliable. This method can provide a continuous readout of pH in isolated liver and skeletal muscle mitochondria and should be useful in assessing the variation of this parameter with mitochondrial activity.

One question not addressed in this study was that of the biochemical properties of the mitochondrial carbonic anhydrase. We do not know the K_m for CO_2, the molecular weight or the turnover number of the enzyme, or whether it is an isozyme unique to mitochondria. Another question left for future investigation is the function of mitochondrial carbonic anhydrase. Liver has an active mitochondrial CO_2 metabolism (3, 4) that is mediated by matrix enzymes; carbonic anhydrase in the matrix would be a logical participant in this metabolism. However, it is a mystery why kidney mitochondria lack the enzyme when it is present in kidney cells in both the cytosol and the plasma membrane (29). The presence of the enzyme in skeletal muscle mitochondria, but not in heart muscle mitochondria, also presents a physiological puzzle, the solution of which should yield much information about intracellular handling of CO_2 in these muscle tissues.

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