The pH Dependence of the Hydration of CO₂ Catalyzed by Carbonic Anhydrase III from Skeletal Muscle of the Cat

STEADY STATE AND EQUILIBRIUM STUDIES

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We have measured the pH dependence of the kinetics of CO₂ hydration catalyzed by carbonic anhydrase III from the skeletal muscle of the cat. Two methods were used: an initial velocity study in which the change in absorbance of a pH indicator was measured in a stopped flow spectrophotometer, and an equilibrium study in which the rate of exchange of ^1⁰₂ between CO₂ and H₂O was measured with a mass spectrometer. We have found that the steady state constants k_Ca²⁺ and k_Ca²⁺ are independent of pH within experimental error in the range of pH 5.0 to 8.5; the rate of release from the enzyme of the oxygen abstracted from substrate HCO₃⁻ in the dehyration is also independent of pH in this range. This behavior is very different from that observed for carbonic anhydrase II for which k_Ca²⁺ and the rate of release of substrate oxygen are very pH-dependent. The rate of interconversion of CO₂ and HCO₃⁻ at equilibrium catalyzed by carbonic anhydrase III is not altered when the solvent is changed from H₂O to 98% D₂O and 2% H₂O. Thus, the interconversion probably proceeds without proton transfer in its rate-limiting steps, similar to isozymes I and II.

A third genetically distinct isozyme of carbonic anhydrase is found in the cytoplasm of skeletal muscle of many mammals (1-5). This isozyme, designated carbonic anhydrase III, has many properties in common with the well studied isozymes carbonic anhydrases I and II which are found in abundance in red cells. All of these isozymes have a molecular weight near 30,000 with one zinc atom/monomer. The complete sequence of bovine carbonic anhydrase III is reported and has 139 out of 260 sequence positions invariant when compared with bovine carbonic anhydrase II (4). Isozymes I and II are monomers but isozyme III can dimerize through formation of a disulfide bond (3).

These three isozymes of carbonic anhydrase have distinctly different activities in the hydration of CO₂ and hydrolysis of esters with isozyme III being the least active (6). The purpose of these studies is to expand this comparison by determining the pH dependence of the CO₂ hydration activity of carbonic anhydrase III from skeletal muscle of the cat. For isozymes I and II, the pH dependence of activity has been informative since the catalysis behaves as if controlled predominantly by a single group in the active site with a pKₙ in the range of 7 to 8, under conditions of physiological ionic strength (7, 8, 10).

We have measured the pH dependence of the catalytic activity of cat muscle carbonic anhydrase III by two methods. The first is a study of the catalytic hydration of CO₂ under initial velocity conditions in which the change in absorbance of a pH indicator is measured in a stopped flow spectrophotometer. The second is a study of the interconversion of CO₂ and HCO₃⁻ at chemical equilibrium in which the exchange of ^1⁰₂ between CO₂ and H₂O is measured with a mass spectrometer. We have found that k_Ca²⁺ and k_Ca²⁺, the steady state parameters for hydration, are independent of pH within experimental error in the range of pH 5.0 to 8.5, and the rate of release from the enzyme of water bearing substrate oxygen is also independent of pH in a similar range. This behavior is in contrast to that observed for isozyme II for which k_Ca²⁺ and the water off-rate are very pH-dependent, although k_Ca²⁺ is also independent of pH for isozyme II (7-10). The ^1⁰₂-exchange method showed that the interconversion of CO₂ and HCO₃⁻ has a solvent deuterium isotope effect of unity for isozyme III and probably proceeds without proton transfer in its rate-limiting steps. It is interesting that both the rate of hydration of CO₂ when catalyzed by isozyme III and the rate of release of substrate oxygen from isozyme III are smaller by a factor of about 300 than that observed when catalyzed by an equal concentration of isozyme II. This suggests that a common property of the catalysis may determine both the catalytic hydration of CO₂ and the release of oxygen from the active site.

EXPERIMENTAL PROCEDURES

Materials and Enzyme—Carbonic anhydrase III was obtained from the hindlimb of fully anesthetized cats in a procedure described by Sanyal et al. (6). This involves double affinity chromatography, first with p-aminomethylbenzenesulfonamide coupled to carboxymethyl (CM)-Bio-Gel A (Bio-Rad) to remove erythrocyte carbonic anhydrases I and II, and second with 2-(4-aminobenzene)-sulfamidol-1,3,4-thiadiazole-5-sulfonamide coupled to CM-Bio-Gel A to isolate carbonic anhydrase III. The second gel was then rinsed clean with Tris sulfate buffer at pH 7.5, following which carbonic anhydrase III was recovered by elution with 0.4 M sodium azide. This isozyme preparation showed a single band in polyacrylamide gel electrophoresis at pH 8.9 and was kept in the monomeric form by storing in a solution of 1 mM dithiothreitol. Human and bovine muscle carbonic anhydrase III were the generous gifts of Professor R. E. Tashian of the University of Michigan. Enzyme concentrations for cat isozyme III were determined using the molar extinction coefficient 4.8 × 10⁴ M⁻¹ cm⁻¹ found experimentally by Sanyal et al. (6). The concentrations of bovine muscle and human muscle carbonic anhydrase III were determined from the extinction coefficients 5.7 × 10⁴ M⁻¹ cm⁻¹ and 6.2 × 10⁴ M⁻¹ cm⁻¹ calculated by Sanyal et al. (6).

Oxygen 18-labeled bicarbonate was prepared by equilibrating...
HCO₃⁻ in ¹⁸O-enriched water (90 atom % ¹⁸O) for 2 days, then distilling off the water. Carbon 13-labeled HCO₃⁻ was prepared by acidifying ¹³C-enriched BaCO₃ (90 atom % ¹³C) and absorbing the resulting ¹³CO₂ into a solution containing KOH in an all glass vacuum system. D₂O (99.8%) was stirred with activated charcoal, then the charcoal was removed by filtration, and the D₂O was distilled. Imidazole was recrystallized twice from hot H₂O.

Stopped Flow Procedure—The initial rates of CO₂ hydration were determined at 10 to 11 °C over a pH range of 5.0 to 8.5 using the pH indicator method of Khalifah (10). A Durrum-Gibson stopped flow spectrophotometer (Model D-110) was used in conjunction with a Nicolet Explorer III oscilloscope. Reaction traces were examined on the oscilloscope and stored on discs. Initial rates were computed for the first 5 to 10% of the reaction using a Hewlett-Packard 9835B computer. Final concentrations of CO₂ were varied from 0.2 to 5.5 mM; at each concentration, the mean initial rate was computed from four reaction traces and the uncatalyzed rate was subtracted to obtain the enzymic rate. The values of K₂₈ and k⁻¹ were calculated using a linear least squares method with n² weights (11, 22), where v is the observed initial velocity.

All experiments were carried out with enzyme which had been passed through Sephadex G-100 to remove dimerized protein. Dithiothreitol was present in enzyme solutions at a final concentration of 0.5 mM in order to maintain the reduced state of the sulphydryl groups. The following buffer-indicator pairs were used: Mes (pK₆ = 6.1) with chlorophenol red (pK₆ = 6.3), Mops (pK₆ = 7.2) with 4-nitrophenol (pK₆ = 7.1), and barbital (pK₆ = 7.9) with phenol red (pK₆ = 7.9). The buffer-indicator pairs were chosen to have nearly identical values of pK₆ for reasons described by Khalifah (10). The reactions were monitored at the wavelength maxima of the basic forms of the indicators. The ionic strengths of the solutions were 0.005 to 0.010 determined predominantly by Na⁺ and the ionized form of buffer; no Na₂SO₄ or K₂SO₄ was used.

Oxygen 18-exchange Procedure—The ¹⁸O exchange between CO₂ and H₂O was measured by mass spectrometry in a procedure identical with that described previously (9). The rate constants for two types of ¹⁸O exchange were measured. The first is the exchange of ¹⁸O between CO₂ and H₂O and occurs because of the hydration-dehydration cycle at chemical equilibrium. The

\[ \text{CO}_2 + H_2O \rightarrow HCOO^{18}O^- + H^+ \rightarrow COO + H^18O \]

first order rate constant \( k \) describes the rate of decrease of the atom fraction of ¹⁸O in CO₂ and is the sum of catalyzed and uncatalyzed parts; \( k = k_{\text{cat}} + k_{\text{uncat}} \). The second exchange is that of ¹³C between ¹³C- and ¹⁸C-containing species of CO₂. The uncatalyzed component of this exchange is due to the reaction between CO₂ and COO⁻ (12); the catalyzed component is due to the labeling of the enzyme (E) with ¹⁸O (9).

\[ \text{HCOO}^{18}O^- + EZNH₂ \rightarrow EZN^{18}O + CO₂ + H₂O \]
\[ EZN^{18}O + ¹³CO₂ + H₂O \rightarrow EZNOH₂ + H^13COO⁻ \]

The first order rate constant \( \phi \) describes the rate of appearance of ¹⁸O in ¹³C-labeled species of CO₂. Here also \( \phi = \phi_{\text{cat}} + \phi_{\text{uncat}} \).

The rate constants \( \theta_{\text{cat}} \) and \( \theta_{\text{uncat}} \) can be related to two steps in the catalysis (9, 13): R₁, the rate at chemical equilibrium of the interconversion of CO₂ and HCOO⁻ and R₂, the rate of release from the enzyme of water or hydroxide ion containing the oxygen abstracted from substrate HCO₂. These rates are described in more detail in Refs. 9 and 13 which also give details of the derivation that results in these equations.

\[ R_{\text{cat}} = \frac{3[CO₂] + [HCOO⁻]}{[CO₂] + [HCOO⁻]} \theta_{\text{cat}} \]

\[ R_{\text{uncat}} = \frac{3[CO₂] + [HCOO⁻]}{[CO₂] + [HCOO⁻]} \theta_{\text{uncat}} \]

Oxygen 18-exchange experiments were performed with a total substrate concentration [CO₂] + [HCOO⁻] = 15 mM and in the presence of sufficient Na₂SO₄ to maintain the total ionic strength of solution at 0.2. Na₂SO₄ has been found to inhibit isozyme II at pH < 7 (14, 15). Dithiothreitol present at 50 mM in the ¹⁸O-exchange experiments was used to prevent dimerization. All ¹⁸O experiments were performed at 25 °C.

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The abbreviations used are: Mes, 4-morpholinesthane-sulfonic acid; Mops, 4-morpholinepropanesulfonic acid.

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**RESULTS**

Steady State Kinetics—Over the range of pH 5.0 to 8.5, the steady state kinetic parameters \( K_{\text{cat}} \) and \( k_{\text{cat}}/K_{\text{cat}} \) for cat muscle carbonic anhydrase III appear independent of pH within experimental uncertainty (Fig. 1). The individual values measured at 10 to 11 °C are \( k_{\text{cat}} = (1.5 \pm 0.5) \times 10^3 \text{ s}^{-1} \) and \( K_{\text{cat}} = 16 \pm 3 \text{ mM} \). These values can be compared to those reported previously at pH 7.5 and 25 °C; \( k_{\text{cat}} = 4.2 \times 10^3 \text{ s}^{-1} \) and \( K_{\text{cat}} = 37 \text{ mM} \) (6). The data in Fig. 1 were obtained using 12.5 mM of the buffers Mes, Mops, or barbital depending on the pH. The same steady state parameters, within experimental error, were obtained when the concentration of Mes and barbital were increased to 25 mM at pH 5.8 and 8.4, respectively. Furthermore, the kinetic constants were not altered by changing the buffer from Mes to Mops at pH 6.9 and from Mops to barbital at pH 7.7.

All experiments were carried out with the monomeric form of cat muscle carbonic anhydrase III obtained by passing the enzyme through Sephadex G-100. Moreover, all solutions contained 0.5 mM dithiothreitol to maintain the reduced form of sulphydryl groups and prevent formation of dimers. We have found that a maximal level of activity can be maintained when the monomeric enzyme is stored in dithiothreitol, but that in its absence a gradual decrease in activity occurs.

**FIG. 1.** The steady state rate constants \( k_{\text{cat}} \) (open symbols) and \( k_{\text{cat}}/K_{\text{cat}} \) (filled symbols) for CO₂ hydration catalyzed by carbonic anhydrase III from cat muscle. The following buffers were present at 12.5 mM: Mes (C, ○); Mops (E, ●); barbital (F, □). Temperature was 10 to 11 °C and enzyme was present at 0.8 to 4 μM.

<table>
<thead>
<tr>
<th>pH</th>
<th>Mes</th>
<th>Mops</th>
<th>Barbital</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.9</td>
<td>11.5</td>
<td>12.7</td>
<td>2.6</td>
</tr>
<tr>
<td>6.2</td>
<td>16.4</td>
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<td>4.9</td>
<td>2.4</td>
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</tr>
<tr>
<td>7.5</td>
<td>2.5</td>
<td>0.4</td>
<td>1.5</td>
</tr>
<tr>
<td>7.9</td>
<td>1.4</td>
<td>0.1</td>
<td>1.2</td>
</tr>
</tbody>
</table>

*Uncorrected pH meter reading.

These solutions also contained 50 mM imidazole. The uncatalyzed values of these rate constants in H₂O at pH 7.4 are \( \theta_{\text{uncat}} = 7.4 \times 10^{-4} \text{ s}^{-1} \) and \( \theta_{\text{cat}} = 1.1 \times 10^{-4} \text{ s}^{-1} \).

Table 1

<table>
<thead>
<tr>
<th>pH(D)</th>
<th>Solvent H₂O</th>
<th>Solvent 98% D₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.9</td>
<td>11.5</td>
<td>12.7</td>
</tr>
<tr>
<td>6.2</td>
<td>16.4</td>
<td>10.3</td>
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<td>0.4</td>
</tr>
<tr>
<td>7.9</td>
<td>1.4</td>
<td>0.1</td>
</tr>
</tbody>
</table>

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1 The abbreviations used are: Mes, 4-morpholinesthane-sulfonic acid; Mops, 4-morpholinepropanesulfonic acid.
neither $[CO_2]$ nor $[HCO_3]$ has exceeded greatly its value of $K_{cat}$, that is, there is no evidence of saturation. $R_{H_2O}$ is independent of substrate concentration in this range (Fig. 4). Table II gives the values of $\theta_{cat}$ and $\phi_{cat}$ from which these results were obtained.

Values of $R_1$ and $R_{H_2O}$ measured from solutions containing bovine muscle and human muscle carbonic anhydrase III are shown in Fig. 5. These data have a pH dependence of $R_1$ and lack of pH dependence of $R_{H_2O}$ very similar to that shown in Table II

**TABLE II**

<table>
<thead>
<tr>
<th>$[CO_3]$</th>
<th>[HCO$_3$]</th>
<th>$\theta_{cat}$</th>
<th>$\phi_{cat}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>11.6</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>6.0</td>
<td>8.5</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>4.5</td>
<td>8.8</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>3.6</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>1.7</td>
<td>8.8</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. The rate of interconversion of $CO_2$ and $HCO_3$, $R_1$, versus uncorrected pH meter reading $pH(D)$ measured in the presence of $2.3 \times 10^{-7}$ M carbonic anhydrase III from cat muscle. Measurements were made in $H_2O$ with 50 mM imidazole (C), in $H_2O$ with no imidazole or other buffer (Δ), and in 98% $D_2O$ with no imidazole or other buffer (Ο). All solutions were maintained at 0.2 total ionic strength by addition of Na$_2$SO$_4$. Temperature was 25°C and $[CO_3]$ + $[HCO_3]$, the total substrate concentration, was constant at 15 mM.

Presumably due to dimerization. The relation between enzyme activity and sulfhydryl status needs further investigation.

**Equilibrium Kinetics—$R_{H_2O}$**

The rate of catalytic interconversion of $CO_2$ and $HCO_3$ at chemical equilibrium, was calculated using Equation 1 from measurements of the rate constants for $^{18}$O-exchange $\theta_{cat}$ and $\phi_{cat}$. Typical results for $\theta_{cat}$ and $\phi_{cat}$ in the presence of $2.3 \times 10^{-7}$ M cat muscle carbonic anhydrase III are given in Table I. In the pH range of these experiments (5.6 to 8.0), the values of $R_1$ obtained at a given pH(D) (uncorrected pH meter reading) were the same within experimental uncertainty when solvent was 100% $H_2O$ or 98% $D_2O$ and 2% $H_2O$ (Ο). Temperature was 25°C and $[CO_3]$ in the presence of 2.3 \times 10^{-7} M $HCO_3$ was present at 2.3 \times 10^{-7} M.

$R_{H_2O}$, the rate of release from the enzyme of water bearing the oxygen abstracted from substrate $HCO_3$, and measured in $H_2O$ (C) and 98% $D_2O$ and 2% $H_2O$ (Ο). Temperature was 25°C with total substrate concentration at 15 mM. No buffers were used. Carbonic anhydrase III was present at 2.3 \times 10^{-7} M.

The dependence of $R_1$ on total substrate, in the range of $[CO_3]$ + $[HCO_3]$ between 2 and 50 mM at pH 6.6, has clearly not reached a plateau at 30 mM (Fig. 4). This indicates that $RH_2O$, the rate of release from the enzyme of water bearing substrate oxygen, was calculated using Equation 2 from measurements of $\theta_{cat}$ and $\phi_{cat}$. $R_{H_2O}$ appears independent of pH in the range of pH 5.6 to 8.0. The rate constants for this process $R_{H_2O} = R_{H_2O}/[E_{cat}]$ exhibit a solvent deuterium isotope effect of 2.4 ± 0.4 in this range of pH (Fig. 3). We have determined that the 50 mM dithiothreitol used in these experiments to prevent dimerization did not inhibit catalytic activity of carbonic anhydrase III under the conditions of these experiments.

The dependence of $R_1$ on total substrate, in the range of $[CO_3]$ + $[HCO_3]$ between 2 and 50 mM at pH 6.6, has clearly not reached a plateau at 30 mM (Fig. 4). This indicates that...
more detail for cat muscle carbonic anhydrase III (Figs. 2 and 3).

**DISCUSSION**

Carbonic anhydrase III from the skeletal muscle of the cat has a turnover number for the hydration of CO$_2$ which is the lowest of the three genetically distinct isozymes known for mammalian carbonic anhydrase. $k_{cat}^{im}$ = (1.5 ± 0.5) $\times$ 10$^4$ s$^{-1}$ at 10 to 11°C, 4.2 $\times$ 10$^3$ s$^{-1}$ at 25°C. This is to be compared with the maximal values for $k_{cat}^{im}$ of 1 $\times$ 10$^5$ s$^{-1}$ for human carbonic anhydrase II and nearly 2 $\times$ 10$^4$ s$^{-1}$ for human carbonic anhydrase I (8, 10) at 25°C. The Michaelis constants for these three isozymes are in magnitude: $K_M^{im}$ is 16 ± 3 mM for cat carbonic anhydrase III, determined in this work, 9 mM for human type II, and 4 mM for human type I isozyme (8, 10). What is new and surprising from this study is that both $k_{cat}^{im}$ and $K_M^{im}$ catalyzed by bovine muscle carbonic anhydrase III also do not vary with pH in a similar pH range. This is in marked contrast to both the human type I and type II isozymes for which $k_{cat}^{im}$ is determined predominantly by ionization of an activity-controlling group with a $pK_a$ near 7.0 for II and above 7.5 for I (10) under conditions of 0.2 ionic strength. $K_M^{im}$ is independent of pH for all isozymes of carbonic anhydrase, types I, II, and III. It is important to note, however, that the Haldane relationship demands that $\frac{k_{cat}^{im}}{K_M^{im}}$ for the dehydration direction be pH-dependent for type III isozyme, provided that HCO$_3^-$ and not H$_2$CO$_3$ is the substrate, as is the case for the type II isozyme. Preliminary results have confirmed this expectation.

The $^{18}$O-exchange data also show the pH-independent nature of the catalytic interconversion of CO$_2$ and HCO$_3^-$ at chemical equilibrium in the range of pH 5.7 to 8. The rate of this interconversion $R_I$ can be described by the expression

$$R_I = \frac{k_{cat}^{im}[E_{cat}][CO_2]}{K_{cat}^{im} + [CO_2]}$$

in which $k_{cat}^{im}$ and $K_{cat}^{im}$ have a different meaning than the steady state turnover number and Michaelis constant (16). $k_{cat}^{im}$ is the maximal rate constant at equilibrium, and $K_{cat}^{im}$ is an apparent binding constant of substrate to enzyme (16).

The data of Fig. 4 are consistent with $k_{cat}^{im} = 2.0 \times 10^4$ s$^{-1}$ and $K_{cat}^{im} > 20$ mM for cat skeletal muscle isozyme III since there is no evidence of saturation at pH 6.6. Since the total substrate [CO$_2$] + [HCO$_3^-$] is 15 mM in Fig. 2, $K_{cat}^{im} > [CO_2]$. Hence, Equation 1 can be approximated as $R_I = \frac{k_{cat}^{im}K_{cat}^{im}}{[CO_2]} [E_{cat}]$. The solid line of Fig. 2 was calculated assuming no pH dependence of $k_{cat}^{im}/K_{cat}^{im}$ and a pH dependence of [CO$_2$] determined by $[H^+][HCO_3^-]/[CO_2] = 4.2 \times 10^{-7}$ M. As shown by Darvey and Koenig et al. (18),

$$\frac{k_{cat}^{im}}{K_{cat}^{im}} = k_{cat}^{im}$$

Hence, the steady state data for hydration and the equilibrium $^{18}$O-exchange data are consistent in showing no pH dependence for $k_{cat}^{im}/K_{cat}^{im}$ in the pH range 5.6 to 8.0. We can also draw the same conclusion from $R_I$ measured in the presence of bovine muscle and human muscle carbonic anhydrase III (Fig. 5). As was also found for human carbonic anhydrase II, the value of $R_I$ in the presence of isozyme III is not changed measurably when 98% D$_2$O and 2% H$_2$O is used as solvent (Fig. 2). Hence the steps which determine the rate of interconversion of CO$_2$ and HCO$_3^-$ catalyzed by these type III isozymes probably do not involve proton transfer in their transition states nor do they involve a group which changes ionization state in the range of pH 5 to 8. The isotope effect of unity on the catalytic interconversion of CO$_2$ and HCO$_3^-$ at equilibrium has been interpreted as consistent with a direct nucleophilic attack of zinc-bound hydroxide on CO$_2$ for human isozymes I and II (16, 19).

The characteristic feature of catalysis of CO$_2$ hydration by isozymes I and II is a pH activity profile indicating an activity-controlling group with a $pK_a$ near 7, which leads us to comment on the origin of the pH-independent profile we have observed for isozyme III. Since it is unlikely that carbonic anhydrase III has an entirely different catalytic mechanism for hydration of CO$_2$, it follows that isozyme III probably has a very pH-dependent $pK_a$ near 7.2. Moreover, the rate constant for release of water bearing substrate oxygen, $R_{H_2O}$, is also independent of pH in the range of pH 6 to 8 (Fig. 3). This is in contrast to the situation with human isozyme II for which $k_{cat}^{im}$ is very pH-dependent (9). Moreover, the rate constant for release of water bearing substrate oxygen is much lower for isozyme III: $R_{H_2O}/[E_{cat}] = 3 \times 10^{-4}$/M s$^{-1}$/2.3 $\times$ 10$^{-7}$ M $= 10^3$ s$^{-1}$ in the absence of buffer (Fig. 3). For human carbonic anhydrase III, the corresponding value is limited by intramolecular proton transfer, has a maximal value of $2 \times 10^3$ s$^{-1}$ at pH 6, and at this pH has a solvent deuterium isotope effect near 8.1. $R_{H_2O}$ does not change as total substrate is varied from 2 to 50 mM at pH 6.6 for type III isozyme (Fig. 4); a similar observation was made for type II isozyme (9).

It may be significant that both $R_I$ and $R_{H_2O}$ have the same magnitude for isozyme III (Figs. 2 and 3), and that $k_{cat}^{im}/K_{cat}^{im}$ and $R_{H_2O}$ are about 300-fold smaller for isozyme III than for isozyme II (9). This and the property that both $k_{cat}^{im}$ and $K_{cat}^{im}$ are independent of pH suggest that the property of isozyme III which determines the smaller value of $R_I$ also determines the smaller value of $R_{H_2O}$. This property could be the decreased nucleophilic character of zinc-bound hydroxide in enzyme III related to the tighter binding of product as well as H$_2$O at the active site.

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**REFERENCES**

Kinetics of Carbonic Anhydrase III from Cat Muscle

Biochem. 59, 253–259
The pH dependence of the hydration of CO2 catalyzed by carbonic anhydrase III from skeletal muscle of the cat. Steady state and equilibrium studies.

C Tu, G Sanyal, G C Wynns and D N Silverman


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