Kinetic Model for the Reversible Hydration of Carbon Dioxide Catalyzed by Human Carbonic Anhydrase II

ARTICLE *in* INDUSTRIAL & ENGINEERING CHEMISTRY RESEARCH · AUGUST 2010

Impact Factor: 2.59 · DOI: 10.1021/ie101338r

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Kinetic Model for the Reversible Hydration of Carbon Dioxide Catalyzed by Human Carbonic Anhydrase II

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Four variants of the two-step Ping Pong mechanism for the reversible hydration of carbon dioxide to bicarbonate catalyzed by free human carbonic anhydrase II (HCA II) in solutions were derived and their goodness-of-fit to match measured initial hydration rates tested. The pseudo (i.e., no central complex) random Quad Quad Iso Ping Pong mechanism with one transitory complex was retained which implied a possible competitive intermolecular proton transfer step by the CO_2/HCO_3^- pair with respect to external buffer. The model suggests this role could be emphasized in product inhibition conditions at high bicarbonate/buffer concentration ratios. A 4-parameter kinetic model was derived to complement the existing single- (intra- or intermolecular) limiting-step models for HCA II catalyzed hydration of CO_2 in which were accounted for altogether the enzyme isomerization and CO_2/HCO_3^- proton transfer via a $[CO_2]\cdot[HCO_3^-]$ coupling, the CO_2/HCO_3^- proton transfer via $[HCO_3^-]^2$ and $[CO_2]\cdot[HCO_3^-]^2$ couplings, and an enzyme−substrate transitory complex via $[CO_2]\cdot[HCO_3^-]\cdot[\text{Buffer}]$ coupling. This model may prove helpful for analysis of CO_2 capture reactor models subject to mixed (intra- or intermolecular) proton-transfer control, intermolecular proton transfer competition by the CO_2/HCO_3^- pair, and large CO_2 conversions.

Introduction

The reversible hydration of carbon dioxide mediated by the zinc−metalloenzyme human carbonic anhydrase II (HCA II) in aqueous solutions has been extensively investigated, primarily from a biochemistry standpoint,[1−3] for HCA II-catalyzed CO_2/HCO_3^- interconversion is vital in many physiological reactions, as well as it is a fairly important reaction for drug design.[4] Recently, emerging ex vivo applications of HCA II for its potential use in CO_2 capture technologies are attracting attention.[5] The main incitement to this interest are the formidably large hydration turnover number, $k_h \approx 10^6\ s^{-1}$, and second-order rate constant, $k_h/K_{CO_2} \approx 10^8\ M^{-1}\cdot s^{-1}$, that Nature endowed this enzyme with to effectuate catalytic hydration of CO_2 near the limits imposed by diffusion encounters in aqueous media.[6] It is therefore very much likely that further engineering developments using such an isozyme will require designing gas−liquid scrubbers with improved mass transfer to accommodate efficiently the short CO_2 hydration time scales related to HCA II.

A prerequisite to this endeavor is to dispose of a kinetic model with a sufficient level of details taking advantage of the abundant body of literature published so far by the biochemists on the CO_2 hydration/dehydration kinetics. To be representative of the multicomponent kinetic evolutions that are to be met in scrubbing units where large CO_2 conversions are desirable and where, therefore, important deviations from the inhibitor-free initial reaction rates inevitably arise, such a detailed model should be calibrated using the broadest possible experimental ranges covered in standard kinetic (i.e., initial reaction rate) measurements.

The most widely accepted mechanism for HCA II hydratase/anhydrase stipulates a two-step reversible CO_2 hydration.[1] The first step implies the conversion of CO_2 (S) into bicarbonate (P) that is spatially sited within the enzyme amphiphilic binding cavity that shelters the zinc-bound hydroxide active site, that is, the catalytic group $Zn(II)-OH^-$. Subsequent release of bicarbonate to the bulk solution is accompanied by a *net* inward displacement of one water molecule[2] (W) changing the enzyme into a $Zn(II)-H_2O$ coordinated form (E_W). To restore the hydroxide-coordinated active catalytic site $Zn(II)-OH^-$ in the enzyme form (E), a second step is set going in which a proton has to be expelled from the E_W coordinated water molecule. However, the proton transfer from the active site all the way to the liquid bulk limits the maximum rate of catalysis.[1] This second step can be further resolved into two main stages: (i) an intramolecular "proton wire"[7] that is rate controlling at high buffer concentration, and (ii) an intermolecular buffer-mediated proton transfer which is rate controlling at low buffer concentration.[1] The proton wire, which consists of a network of coordinated water molecules of the amino-acids lining the HCA II binding cavity, relays first the proton from $Zn(II)-H_2O$ to the imidazole ring of (the internal proton shuttle) histidine 64 residue (His64) sitting by the periphery of the cavity. This stage is depicted as an isomerization ($E_W \rightleftharpoons {}_HE$) and corresponds to a "domestic" deprotonation in which the E_W catalytic group is deprotonated at the expense of protonation of the His64 proton-transfer group leading to enzyme isomerized form ${}_HE$.[8] Because of the distinctive orientational dynamics of His64,[9] the proton from ${}_HEis$ externalized in subsequent stage (ii) toward the bulk solution via protonation of the basic form (B) of an external buffer. This completes the catalytic cycle by restoring the active form (E) to initiate a new cycle for catalytic hydration. Since bicarbonate product is required to leave before the buffer substrate interacts with the enzyme, the enzyme oscillates between more than one stable form, in addition to its isomerization. Such a mechanism is christened the Iso Ping Pong mechanism.[10]

Steiner et al.[8,11] investigated the kinetics of CO_2 reversible hydration catalyzed by HCA II mainly under conditions that emphasized the (intramolecular) proton wire as rate-controlling using sufficiently high buffer concentrations. Hydration rates were reported up to large concentrations of product inhibitor[8]

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10.1021/ie101338r

Table 1. Kinetic Data Ranges Covered in HCA II Catalyzed Reversible Hydration of CO_2[a]

pH	[7.8−8.9]
[S], mM	[0.24−17]
[P], mM	[0−203]
[B], mM	[1−42]
[BH^+], mM	[0.5−22]
[E_0], nM	[69−210]
[P]/[B]	[0−26]
S/P pair pK_{a1} (mol/L)[b]	5.97
$S + W \rightleftharpoons P + H^+$; $K_{a1} = ([P]\,[H^+])/[S]$	
Ka1/Ka2	[13.5−169.6]
pK_E (mol/L)[c]	7.1
proton-transfer group acid dissociation constant, $_HE$	
$_HE \rightleftharpoons E + H^+$; $K_E = ([E]\,[H^+])/[_HE]$	
catalytic group acid dissociation constant, E_W	≈7.1
$E_W \rightleftharpoons E + H^+$; $K_E = ([E]\,[H^+])/[E_W]$	
buffer pK_{a2} (mol/L)	
1,2-dimethylimidazole (1,2-DMI)	8.2
triethanolamine (TEA)	7.76
2,2-diethylmalonate (2,2-DEM)	7.1
$B + H^+ \rightleftharpoons BH^+$; $K_{a2} = ([B]\,[H^+])/[BH^+]$	

[a] Data from Rowlett and Silverman,[12] Steiner et al.,[8] and Jonsson et al.[13] [b] After Soli and Byrne.[16] [c] Silverman and Lindskog.[1]

($[HCO_3^-]_{max} = 200$ mM) but where interference with buffer in proton transfer via proton−acceptor bicarbonate was ignored in the analysis. This seems justified considering their [P]/[B] ratios ranged from 0.7 to 7 and $K_{a1}/K_{a2} \approx 169$ (Table 1). Two kinetic model versions were proposed: (1) one in which a competitive product inhibition of CO_2 hydration was accounted for via a three-parameter Michaelis−Menten reversible kinetic model with pH-dependent hydration turnover number and a couple of (pH-indifferent) Michaelis constants for CO_2 and bicarbonate,[11] (2) and a second one in which the role of isomerization ($E_W \rightleftharpoons {}_HE$, [S] · [P] coupling) was accounted for by including an additional parameter evolving the kinetic expression into a 4-parameter model.[8]

Rowlett and Silverman[12] specifically measured the CO_2 hydration initial rate patterns at sufficiently low buffer concentrations to displace hydration into a regime where the intermolecular proton transfer became rate determining. Understandably, enzyme isomerization was not parametrized in their kinetic model (no [S] [P] coupling). However, the role of the interaction between the external buffer and the internal proton shuttle His64 for proton transfer was explicitly accounted for in the mechanism by means of a Brønsted-type rate constant. Their kinetics was expressed in the form of a 4-parameter model involving a buffer-dependent hydration turnover rate, a Michaelis constant for carbon dioxide, a Michaelis constant for the unprotonated buffer, and an apparent bicarbonate inhibition constant. The buffer Michaelis and bicarbonate inhibition constants took on different values for each buffer but, unlike the turnover rate, were not correlated to the reaction phenomenology. Product inhibition on CO_2 hydration was assessed up to $[HCO_3^-]_{max} = 50$ mM. It is worth noting that the mechanistic event of proton shuttling via His64 and bicarbonate in lieu of the proton-acceptor buffer form was not considered in spite of [P]/[B] ratios as high as 26.

To the best of our knowledge, these kinetic models are the most general ones pertaining to the reversible HCA II catalyzed hydration/dehydration of carbon dioxide in aqueous media. In the perspective of achieving large carbon dioxide abatement as a target for CO_2 capture, it is crucial to anticipate how far the enzymic hydration kinetics would move away from the maximum turnover conditions as a result of build-up of product inhibitor and depletion of the proton-acceptor buffer form. The purpose of the present study is, therefore, to appraise three questions: (1) Is any of these kinetic models general enough to be helpful for analyzing intermediate instances characterized by mixed intra/intermolecular proton-transfer control? (2) Is there any evidence as to how the intermolecular proton transfer by the CO_2/HCO_3^- pair could interfere competitively with B/BH^+ external buffer pair? (3) To which extent enzyme isomerization and/or intermolecular CO_2/HCO_3^--subtended proton transfer via $[CO_2]\cdot[HCO_3^-]$ coupling, CO_2/HCO_3^--subtended proton transfer via $[HCO_3^-]^2$ and $[CO_2]\cdot[HCO_3^-]^2$ couplings, and enzyme−substrate transitory complex via $[CO_2]\cdot[HCO_3^-]\cdot[B]$ coupling are important kinetic features?

Discussion

Current Kinetic Models. Kinetic data were collected from the works of Steiner et al.,[8] Jonsson et al.,[13] and Rowlett and Silverman.[12] These were the only sources for which the kinetic experiments were backed with sufficient information in the original references to conduct detailed comparisons and kinetic calculations. Table 1 describes the kinetic data for which the HCA II-catalyzed reversible hydration for carbon dioxide was obtained. The full set of experimental kinetic data is provided as an appendix in the Supporting Information.

Figures 1a−c illustrate the parity plots of calculated versus measured initial CO_2 hydration rates by human carbonic anhydrase II for the kinetic models without enzyme isomerization (Figure 1a) of Steiner et al.,[11] with enzyme isomerization (Figure 1b) of Steiner et al.[8] and with intermolecular limiting step (Figure 1c) of Rowlett and Silverman.[12] Furthermore, the ranking of these models was attempted through a comparison of the absolute average relative error:

$$\mathrm{AARE} = \frac{1}{N}\sum_1^N \left| \frac{\mathrm{measured}\dfrac{1\mathrm{d}[P]}{[E_0]\mathrm{d}t} - \mathrm{calculated}\dfrac{1\mathrm{d}[P]}{[E_0]\mathrm{d}t}}{\mathrm{measured}\dfrac{1\mathrm{d}[P]}{[E_0]\mathrm{d}t}} \right| \quad (1)$$

The intermolecular His64/buffer proton exchanges being assumed fast in both Steiner et al.[8,11] kinetic models led to an overprediction of the initial hydration rates of Rowlett and Silverman,[12] see Figure 1a and b. Some of Jonsson et al.[13] measurements, in particular those carried out at low buffer concentration ([B] < 10 mM), exposed the same overestimation (Figure 1a, b), in accordance with the authors' observations of participation of buffer in the catalytic mechanism of carbonic anhydrase. As indicated in Table 2, the AARE for the whole data set was rather large, amounting to 75.4% and 82.4% for the 3-parameter and the 4-parameter model, respectively. Selecting successively Steiner et al.[8] data at $[P] \neq 0$ and Rowlett and Silverman[12] at $[P] \neq 0$ led to AARE of 22.8% and 149%, respectively, using the 3-parameter kinetic model; whereas the 4-parameter model version gave, respectively, 4.6% and 188%. Hence, Steiner et al. models are not appropriate to capture the kinetic hydration behavior in the buffer low-concentration region when product inhibition is enabled. Furthermore, predictability by the 4-parameter model in the low buffer concentration range was even degraded through inclusion of a $[CO_2]\cdot[HCO_3^-]$ coupling to account for isomerization. However, as will be discussed later, this does not rule out the existence of S/P coupling terms in the kinetic model that would influence the hydration rates at low buffer concentrations by invoking additional mechanistic features, including enzyme isomerization.

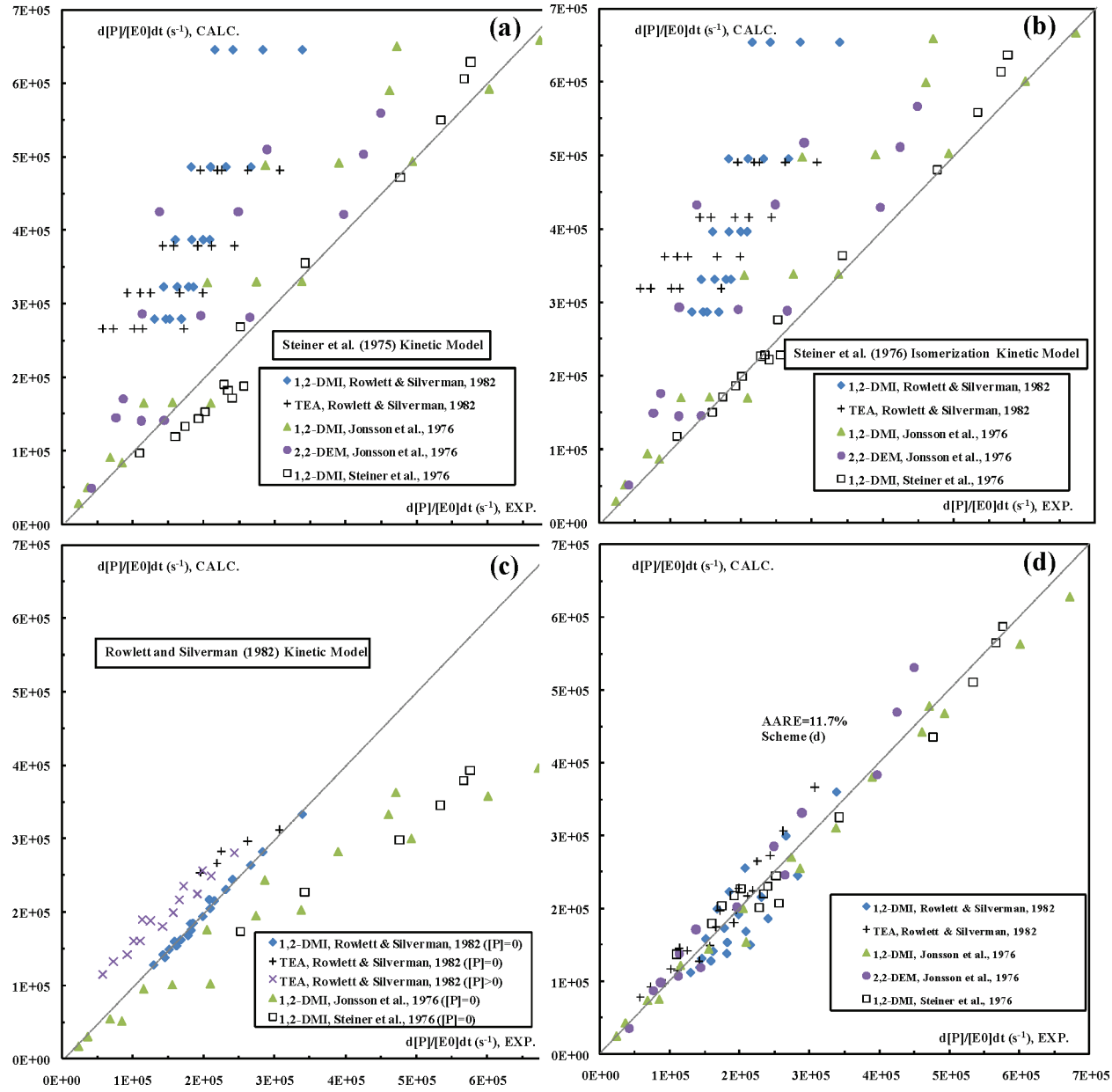

Figure 1. Parity plot of calculated versus measured initial CO_2 hydration rate by human carbonic anhydrase II: Kinetic model (a) Steiner et al.,[11] (b) Steiner et al.,[8] (c) Rowlett and Silverman,[12] and (d) Scheme d, eq 14, Table 6.

Table 2. Ranking of Kinetic Models Based on AARE

kinetic model	AARE (%)
Steiner et al.,[8] with enzyme isomerization 4 parameters	82.4 (4.6[a,b], 188[a,c])
Steiner et al.,[11] no enzyme isomerization 3 parameters	75.4 (22.8[a,b], 149[a,c])
Rowlett and Silverman,[12] 3 + 1 parameters	22.7 (43.4[a,c])
ordered pseudo Ter Bi Iso Ping Pong 3 parameters, eq 11, Table 3 (scheme a)	18.6 (34.5[a,d])
Ordered pseudo Ter Bi Iso Ping Pong 4 parameters, eq 13, Table 5 (scheme c)	16.2 (28.0[a,d])
random pseudo Quad Quad Iso Ping Pong 4 parameters, eq 12, Table 4 (scheme b)	18.4
random pseudo Quad Quad Iso Ping Pong 4 parameters, eq 14, Table 6 (scheme d)	11.7 (13.2[a,d])

[a] Only initial CO_2 hydration rates in presence of product inhibition, [P] ≠ 0. [b] Steiner et al. data.[8] [c] Rowlett and Silverman[12] data. [d] Both a + b.

Mirroring in opposite the trends featured by the previous kinetic models, Rowlett and Silverman[12] kinetic model by stressing the slowness of the intermolecular proton exchanges led to an underestimation of Steiner et al.[8] and Jonsson et al.[13] measured hydration rates except for the ones measured by Jonsson et al.[13] at the lowest buffer concentration ([B] < 10 mM), see Figure 1c. The AARE for the whole data set amounted to 22.7% but inflated to 43.4% if only the hydration rates obtained in the presence of product inhibitor are considered (Table 2). Note that Steiner et al.[8] measurements for [P] ≠ 0 could not be compared to this kinetic model because an apparent bicarbonate inhibition constant was lacking.[12] Also, the positively biased hydration rates calculated in the case of triethanolamine buffer (see Figure 1c) cannot be ascribed solely to the approximation of (CO_2-independent) bicarbonate inhibition constant as the same deviation was revealed for the rates at [P] = 0. As recognized by Rowlett and Silverman,[12] triethanolamine Michaelis constant and the corresponding hydration turnover that resulted from data fitting at [P] = 0 did not work well in the [P] ≠ 0 conditions. Unlike the excellent fit achieved with 1,2-dimethylimmidzaole buffer (Figure 1c) in the absence

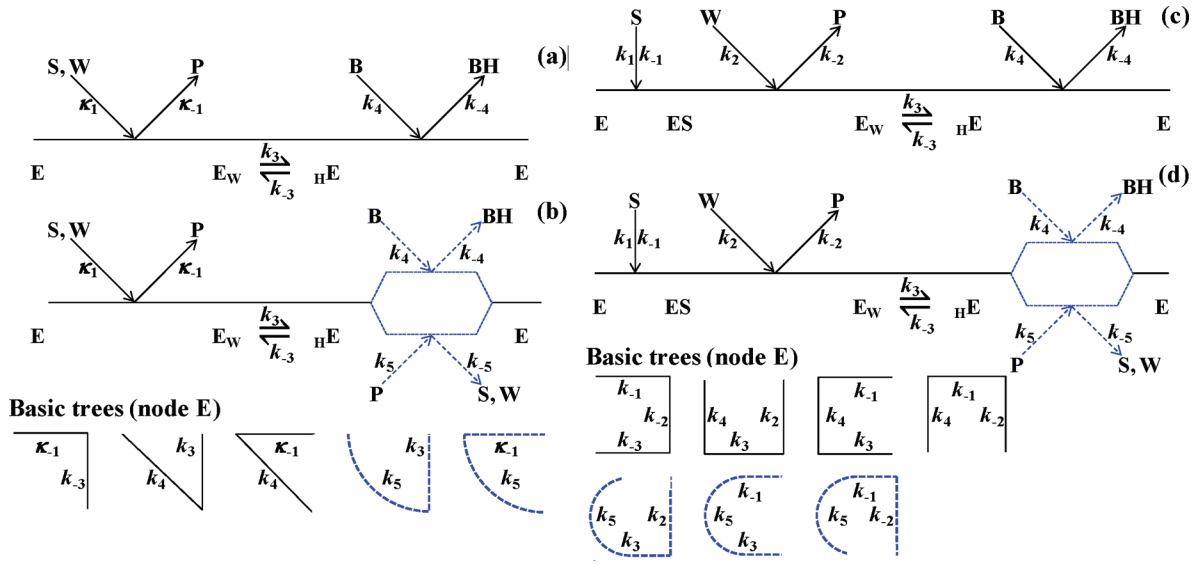

Figure 2. Ordered Ter Bi (a,c) and random Quad Quad (b,d) Pseudo Iso Ping Pong catalytic mechanism for reversible CO_2 hydration by human carbonic anhydrase II: No transitory complex (a, b) and one transitory complex, ES (c, d).

Table 3. Kinetic Model for Reversible CO_2 Hydration-Ordered Pseudo Ter Bi Iso Ping Pong Catalytic (0-Transitory-Complex) Mechanism (Scheme a)

• Rate equation (King and Altman's[14] method based on rate constants)

$$\frac{1}{[E_0]}\frac{d[P]}{dt} = \frac{k_3k_4\left([S][B] - \frac{K_{a2}}{K_{a1}}[P][BH^+]\right)}{k_3\left(2[S] + \frac{K_E}{K_{a1}}[P]\right) + \frac{k_3}{\kappa_1[W]}k_4\left([B] + 2\frac{K_{a2}}{K_E}[BH^+]\right) + k_4[S][B] + k_4\left(\frac{K_E}{K_{a1}}[B] + \frac{K_{a2}}{K_{a1}}[BH^+]\right)[P]}$$

• Rate equation (Cleland's[10] method based on estimable kinetic constants)

$$\frac{1}{[E_0]}\frac{d[P]}{dt} = \frac{k_h\left([S][B] - \frac{K_{a2}}{K_{a1}}[P][BH^+]\right)}{K_B[S] + K_S[B] + \frac{K_BK_E}{2K_{a1}}[P] + 2K_S\frac{K_{a2}}{K_E}[BH^+] + [S][B] + \frac{K_{a2}}{K_E}[P][BH^+] + \frac{K_E}{K_{a1}}[B][P]} \quad (11)$$

• Hydration and dehydration (turnover) rate constants

$$k_h = k_d + k_3$$

• S, P, B, and BH^+ apparent (Michaelis) binding constants

$$K_S = \frac{\kappa_3}{\kappa_1[W]}; K_B = 2\frac{k_3}{k_4} = 2\frac{k_3}{k_\infty}\frac{K_E + K_{a2}}{K_E} = K_{B\infty}\frac{K_E + K_{a2}}{K_E}$$

$$K_P = 2\frac{K_{a1}}{K_E}\frac{k_3}{\kappa_1[W]} = 2\frac{K_{a1}}{K_E}K_S; K_{BH} = \frac{K_B}{2}\frac{K_E}{K_{a2}} = \frac{k_3}{k_4}\frac{K_E}{K_{a2}} = \frac{k_3}{k_\infty}\frac{K_E + K_{a2}}{K_{a2}} = K_{BH\infty}\frac{K_E + K_{a2}}{K_{a2}}$$

• Enzyme distribution equations

$$[E] \propto k_3k_4[B] + \frac{K_E}{K_{a1}}\kappa_1[W]k_3[P] + \frac{K_E}{K_{a1}}\kappa_1[W]k_4[B][P]$$

$$[E_W] \propto \kappa_1[W]k_3[S] + \frac{K_{a2}}{K_E}k_3k_4[BH^+] + \kappa_1[W]k_4[S][B]$$

$$[_HE] \propto \kappa_1[W]k_3[S] + \frac{K_{a2}}{K_E}k_3k_4[BH^+] + \frac{K_{a2}}{K_{a1}}\kappa_1[W]k_4[P][BH^+]$$

of product inhibitor, the model shows limitations at handling the presence of P at low buffer concentrations.

These comparisons conclude that none of the above models can be relied upon as a general kinetic model for describing the behavior of CO_2 hydration kinetics, a fortiori to capture kinetics with mixed intra/intermolecular proton-transfer control. Hence, the answer to the first formulated question above is no. Steiner et al.[8,11] models are not applicable when intermolecular proton transfer is rate-limiting especially in the presence of product inhibition ([P]≠0) and low buffer concentrations. Whereas, Rowlett and Silverman[12] model does not extrapolate robustly toward high buffer concentrations nor does it account adequately for the presence of inhibitor at low buffer concentrations.

New Kinetic Model. Four variants of the two-step Ping Pong mechanism for the reversible hydration of CO_2 catalyzed by free human carbonic anhydrase II (HCA II) in solutions were derived and their goodness-of-fit to match the compiled hydra-

Table 4. Kinetic Model for Reversible CO_2 Hydration-Random Pseudo Quad Quad Iso Ping Pong Catalytic (0-Transitory-Complex) Mechanism (Scheme b)

• Rate equation (King and Altman's[14] method based on rate constants)

$$\frac{1}{[E_0]}\frac{d[P]}{dt} = \frac{\left([S][B] - \frac{K_{a2}}{K_{a1}}[P][BH^+]\right)\left(k_3k_4\left(1 + \frac{K_{a1}k_5}{K_E\kappa_1[W]}\right) + k_4k_5[P]\right)}{K_3\left(1 + \frac{K_{a1}k_5}{K_E\kappa_1[W]}\right)\left(2[S] + \frac{K_E}{K_{a1}}[P]\right) + \frac{k_3}{\kappa_1[W]}k_4\left([B] + 2\frac{K_{a2}}{K_E}[BH^+]\right) + k_4[S][B] + 2k_5[S][P] + k_4\left(\frac{K_E}{K_{a1}}[B] + \frac{K_{a2}}{K_{a1}}[BH^+]\right)[P] + \frac{K_E}{K_{a1}}k_5[P]^2}$$

• Rate equation (Cleland's[10] method based on estimable kinetic constants)

$$\frac{1}{[E_0]}\frac{d[P]}{dt} = \frac{k_h\left([S][B] - \frac{K_{a2}}{K_{a1}}[P][BH^+]\right)\left(1 + \frac{[P]}{K_{iP,1}}\right)}{K_B[S] + K_S[B] + \frac{K_BK_E}{2K_{a1}}[P] + 2K_S\frac{K_{a2}}{K_E}[BH^+] + [S][B] + \frac{K_{a2}}{K_E}[P][BH^+] + \frac{K_B}{K_{iP,1}}[S][P] + \frac{K_E}{K_{a1}}[B][P] + \frac{1K_BK_E}{2K_{iP,1}K_{a1}}[P]^2} \quad (12)$$

• Hydration and dehydration (turnover) rate constants

$$k_h = k_3\left(1 + \frac{K_{a1}}{K_E}\frac{k_5}{\kappa_1[W]}\right); k_d = k_h$$

• S, P, B, and BH^+ apparent (Michaelis) binding constants

$$K_S = \frac{k_3}{\kappa_1[W]}; K_B = 2\frac{k_3}{k_4}\left(1 + \frac{K_{a1}}{K_E}\frac{k_5}{\kappa_1[W]}\right) = 2\frac{k_3}{k_\infty}\frac{K_E + K_{a2}}{K_E}\left(1 + \frac{K_{a1}}{K_E}\frac{k_5}{\kappa_1[W]}\right) = K_{B\infty}\frac{K_E + K_{a2}}{K_E}$$

$$K_P = 2\frac{K_{a1}}{K_E}\frac{k_3}{\kappa_1[W]} = 2\frac{K_{a1}}{K_E}K_S; K_{BH} = \frac{K_B}{2}\frac{K_E}{K_{a2}} = \frac{k_3}{k_4}\frac{K_E}{K_{a2}}\left(1 + \frac{K_{a1}}{K_E}\frac{k_5}{\kappa_1[W]}\right) = \frac{k_3}{k_\infty}\frac{K_E + K_{a2}}{K_{a2}}\left(1 + \frac{K_{a1}}{K_E}\frac{k_5}{\kappa_1[W]}\right) = K_{BH\infty}\frac{K_E + K_{a2}}{K_{a2}}$$

• P apparent inhibition constants

$$K_{iP,1} = \frac{k_3}{k_5}\left(1 + \frac{K_{a1}}{K_E}\frac{k_5}{\kappa_1[W]}\right)$$

$$K_{iP,2} = \frac{K_{a1}}{K_E}\frac{k_3}{\kappa_1[W]} = \frac{K_{a1}}{K_E}K_S$$

• Enzyme distribution equations

$$[E] \propto k_3k_4[B] + k_3\left(\frac{K_E}{K_{a1}}\kappa_1[W] + k_5\right)[P] + \frac{K_E}{K_{a1}}\kappa_1[W]k_5[P]^2 + \frac{K_E}{K_{a1}}\kappa_1[W]k_4[B][P]$$

$$[E_W] \propto \frac{K_{a1}}{K_E}k_3\left(\frac{K_E}{K_{a1}}\kappa_1[W] + k_5\right)[S] + \frac{K_{a2}}{K_E}k_3k_4[BH^+] + \kappa_1[W]k_4[S][B] + \kappa_1[W] + k_5[S][P]$$

$$[{}_HE] \propto \frac{K_{a1}}{K_E}k_3\left(\frac{K_E}{K_{a1}}\kappa_1[W] + k_5\right)[S] + \frac{K_{a2}}{K_E}k_3k_4[BH^+] + \frac{K_{a2}}{K_{a1}}\kappa_1[W]k_4[P][BH^+] + \kappa_1[W]k_5[S][P]$$

tion rates analyzed. The pseudo (i.e., no central complex) ordered Ter Bi Iso Ping Pong mechanisms assumed three substrates (S, W, B) and two products (P, BH^+), alternatively accounting for one or no transitory complex (Figure 2a, c). Likewise, four substrates (S, W, B, P) and as many products (P, BH^+, S, and W), were involved in the pseudo-random Quad Quad Iso Ping Pong mechanisms (Figures 2b, d). The basic trees for the ordered (shown only as solid lines) and random mechanisms are also shown in Figure 2.

The net forward enzymic reaction rates for the random mechanisms are written as

Scheme b

$$\frac{d[P]}{dt} = k_1[W][S][E] - k_{-1}[P][E_W] + [W]k_{-5}[S][E] - k_5[P][{}_HE] \quad (2)$$

Scheme d

$$\frac{d[P]}{dt} = k_1[W][ES] - k_{-2}[P][E_W] + [W]k_{-5}[S][E] - k_5[P][{}_HE] \quad (3)$$

A helpful method put forward by King and Altman[14] was used to develop the *rate-constant* forms of the rate equations for each mechanism by expressing them solely as a function of substrates, products and total enzyme ($[E_0]$) concentrations. We strived to build these mechanisms so as to keep as many parameters (and no more), that is, 3 or 4, as implied in the kinetic models discussed previously. Despite a reduced set of parameters, not all of the rate constants are individually identifiable. Therefore, the rate equations were alternatively formulated in *kinetic-constant* forms by clustering the rate constants into forward and reverse turnovers and Michaelis and inhibition constants.[10] Both rate-constant and kinetic-constant forms of these rate equations are summarized in Tables 3−6, along with expressions for the hydration and dehydration turnover numbers, k_h and k_d, the apparent Michaelis constants, K_S, K_P, K_B, and K_{BH}, the apparent bicarbonate inhibition constants, $K_{iP,j}$, and the enzyme distributions for E, E_W, $_HE$, and ES. The ordered (3-parameter) Ter Bi and random (4-parameter) Quad Quad Pseudo Iso Ping Pong mechanisms with no transitory complex, ES, are summarized in Tables 3 and 4, respectively; while the ordered (4-parameter) Ter Bi and random (4-parameter) Quad Quad Pseudo Iso Ping Pong mechanisms

Table 5. Kinetic Model for Reversible CO_2 Hydration-Ordered Pseudo Ter Bi Iso Ping Pong Catalytic (1-Transitory-Complex) Mechanism (Scheme c)

• Rate equation (King and Altman's[14] method based on rate constants)

$$\frac{1}{[E_0]}\frac{d[P]}{dt} = \frac{k_3\left([S][B] - \frac{K_{a2}}{K_{a1}}[P][BH^+]\right)}{\frac{K_E k_3}{K_{a1}k_4}\left(2\frac{K_{a1}}{K_E}[S] + [P]\right) + \frac{k_3}{k_1}\left(1 + \frac{k_{-1}}{k_2[W]}\right)\left([B] + 2\frac{K_{a2}}{K_E}[BH^+]\right) + \left(1 + \frac{k_3}{k_2[W]}\right)[S][B] + \frac{k_3 k_1 K_E}{k_{-1}k_4 K_{a1}}[S][P] + \left(\frac{K_E}{K_{a1}}[B] + \frac{K_{a2}}{K_{a1}}\left(\frac{k_3}{k_{-1}} + 1\right)[BH^+]\right)[P] + \frac{K_E k_1}{K_{a1}k_{-1}}[S][B][P]}$$

• Rate equation (Cleland's[10] method based on estimable kinetic constants)

$$\frac{1}{[E_0]}\frac{d[P]}{dt} = \frac{k_h\left([S][B] - \frac{K_{a2}}{K_{a1}}[P][BH^+]\right)}{K_B[S] + K_S[B] + \frac{K_B K_E}{2K_{a1}}[P] + 2K_S\frac{K_{a2}}{K_E}[BH^+] + [S][B] + 2\frac{K_S K_{a2}}{K_P K_E}[P][BH^+] + \frac{K_B}{K_{iP,1}}[S][P] + \frac{K_S}{K_{iP,2}}[B][P] + \frac{1}{K_{iP,5}}[S][B][P]} \quad (13)$$

• Hydration and dehydration (turnover) rate constants

$$K_h = \frac{k_3}{1 + \frac{k_3}{k_2[W]}} \approx k_3; k_d = \frac{k_h}{2}\frac{K_P}{K_S}\frac{K_E}{K_{a1}} = \left(\frac{1}{k_3} + \frac{1}{k_{-1}}\right)^{-1}$$

• S, P, B, and BH^+ apparent (Michaelis) binding constants

$$K_S = \frac{k_3}{k_1}\frac{1 + \frac{k_{-1}}{k_2[W]}}{1 + \frac{k_3}{k_2[W]}} \approx \frac{k_3}{k_1}; K_B = \frac{2}{k_4}\frac{k_3}{1 + \frac{k_3}{k_2[W]}} \approx \frac{2k_3}{k^\infty}\frac{K_E + K_{a2}}{K_E} = K_{B\infty}\frac{K_E + K_{a2}}{K_E}; K_P = 2\frac{K_{a1}}{K_E}\frac{k_3}{k_1}\frac{1 + \frac{k_{-1}}{k_2[W]}}{1 + \frac{k_3}{k_{-1}}} \approx \frac{2}{k_1\left(\frac{1}{k_3} + \frac{1}{k_{-1}}\right)}\frac{K_{a1}}{K_E}$$

$$K_{BH} = \frac{K_B}{4}\frac{K_P}{K_S}\frac{K_E}{K_{a1}}\frac{K_E}{K_{a2}} = \frac{1}{k_4}\frac{K_E}{K_{a2}}\left(\frac{1}{k_3} + \frac{1}{k_{-1}}\right)^{-1} \approx \frac{1}{k_\infty}\frac{K_E + K_{a2}}{K_{a2}}\left(\frac{1}{k_3} + \frac{1}{k_{-1}}\right)^{-1} = K_{BH\infty}\frac{K_E + K_{a2}}{K_{a2}}$$

• P apparent inhibition constants

$$K_{iP,1} = 2\frac{K_{a1}}{K_E}\frac{k_{-1}}{k_1}$$

$$K_{iP,2} = \frac{K_{a1}}{K_E}\frac{k_3}{k_1}\left(1 + \frac{k_{-1}}{k_2[W]}\right) \approx \frac{K_{a1}}{K_E}\frac{k_3}{k_1} = \frac{K_{a1}}{K_E}K_S; K_{iP,5} = \frac{K_{a1}}{K_E}\frac{k_{-1}}{k_1}\left(1 + \frac{k_3}{k_2[W]}\right) \approx \frac{K_{a1}}{K_E}\frac{k_{-1}}{k_1}\frac{K_{iP,1}}{2}$$

• Enzyme distribution equations

$$[E] \propto k_3 k_4\left(1 + \frac{k_{-1}}{k_2[W]}\right)[B] + \frac{K_E}{K_{a1}}k_1 k_3[P] + \frac{K_E}{K_{a1}}k_1 k_4[B][P]$$

$$[ES] \propto k_1 k_4\frac{k_3[S][B]}{k_2[W]} + \frac{K_E}{K_{a1}}k_1 k_3\frac{k_1}{k_{-1}}[S][P] + \frac{K_{a2}}{K_{a1}}k_3 k_4\frac{k_1}{k_{-1}}[BH^+][P] + \frac{K_E}{K_{a1}}k_1 k_4\frac{k_1}{k_{-1}}[S][B][P]$$

$$[E_W] \propto k_1 k_3[S] + \frac{K_{a2}}{K_E}k_3 k_4\left(1 + \frac{k_{-1}}{k_2[W]}\right)[BH^+] + k_1 k_4[S][B]$$

$$[{}_HE] \propto k_1 k_3[S] + \frac{K_{a2}}{K_E}k_3 k_4\left(1 + \frac{k_{-1}}{k_2[W]}\right)[BH^+] + \frac{K_{a2}}{K_{a1}}k_1 k_3[P][BH^+]$$

assuming one transitory complex, ES, are given, respectively, in Tables 5 and 6.

The rate constant $k_2[W]$ for the solvation step in schemes c and d turned out to be not identifiable on the basis of the collected kinetic data set. Hence, solvation in the enzyme binding cavity was assumed to proceed much faster than the unproductive decomposition of the transitory complex, ES, that is, $k_{-1} \ll k_2[W]$, as well as the subsequent enzyme isomerization, that is, $k_3 \ll k_2[W]$.

The rate constants for the reverse reactions in schemes a to d (Figure 2) can be resolved in terms of the forward rate constants in combination with the equilibrium constants given in Table 1. Hence, since a single pK_E value was assumed for the endogenous residue proton-transfer group His64 in the enzyme form ${}_HE$, and for the metal-bound catalytic group in the enzyme form, E_W, it can easily be shown that

$$k_{-3} = k_3 \quad (4)$$

Table 6. Kinetic Model for Reversible CO_2 Hydration-Random Pseudo Quad Quad Iso Ping Pong Catalytic (1-Transitory-Complex) Mechanism (Scheme d)

• Rate equation (King and Altman's[14] method based on rate constants)

$$\frac{1}{[E_0]}\frac{d[P]}{dt} = \frac{\left([S][B] - \frac{K_{a2}}{K_{a1}}[P][BH^+]\right)\left(\frac{K_{a1}k_3}{K_E k_1}k_4\left(\frac{K_E}{K_{a1}}k_1 + k_5\left(1 + \frac{k_{-1}}{k_2[w]}\right)\right) + k_4k_5[P]\right)}{\frac{k_3}{k_1}\left(\frac{K_E}{K_{a1}}k_1 + k_5\left(1 + \frac{k_{-1}}{k_2[w]}\right)\right)\left(2\frac{K_{a1}}{K_E}[S] + [P]\right) + \frac{k_3}{k_1}k_4\left(1 + \frac{k_{-1}}{k_2[w]}\right)\left([B] + 2\frac{K_{a2}}{K_E}[BH^+]\right) + k_4\left(1 + \frac{k_3}{k_2[w]}\right)[S][B] + \left(2k_5 + \frac{k_3k_1}{k_1k_{-1}}\left(\frac{K_E}{K_{a1}}k_1 + k_5\left(1 + \frac{k_{-1}}{k_2[w]}\right)\right)\right)[S][P] + k_4\left(\frac{K_E}{K_{a1}}[B] + \frac{K_{a2}}{K_{a1}}\left(\frac{k_3}{k_{-1}} + 1\right)[BH^+]\right)[P] + \frac{K_E}{K_{a1}}k_5[P]^2 + \frac{K_Ek_1}{K_{a1}k_{-1}}k_5[S][P]^2 + \frac{K_Ek_1}{K_{a1}k_{-1}}k_4[S][B][P]}$$

• Rate equation (Cleland's[10] method based on estimable kinetic constants)

$$\frac{1}{[E_0]}\frac{d[P]}{dt} = \frac{k_h\left([S][B] - \frac{K_{a2}}{K_{a1}}[P][BH^+]\right)\left(1 + \frac{[P]}{K_{iP,3}}\right)}{k_B[S] + K_S[B] + \frac{K_BK_E}{2K_{a1}}[P] + 2K_S\frac{K_{a2}}{K_E}[BH^+] + [S][B] + 2\frac{K_SK_{a2}}{K_PK_E}[P][BH^+] + \frac{K_B}{K_{iP,1}}[S][P] + \frac{K_S}{K_{iP,2}}[B][P] + \frac{1K_BK_E}{2K_{iP,3}K_{a1}}[P]^2 + \frac{K_B}{K_{iP,1}K_{iP,4}}[S][P]^2 + \frac{1}{K_{iP,5}}[S][B][P]} \quad (14)$$

• Hydration and dehydration (turnover) rate constants

$$k_h = \frac{K_{a1}}{K_E}\frac{k_3}{k_1}\frac{\frac{K_E}{K_{a1}}k_1 + k_5\left(1 + \frac{k_{-1}}{k_2[w]}\right)}{1 + \frac{k_3}{k_2[w]}} \approx \frac{K_{a1}}{K_E}\frac{k_3}{k_1}\left(\frac{K_E}{K_{a1}}k_1 + k_5\right); k_d = \frac{k_h}{2}\frac{K_P}{K_S}\frac{K_E}{K_{a1}} = \frac{K_{a1}}{K_E}\frac{k_3}{k_1}\frac{\frac{K_E}{K_{a1}}k_1 + k_5\left(1 + \frac{k_{-1}}{k_2[w]}\right)}{1 + \frac{k_3}{k_{-1}}} \approx \frac{K_{a1}}{K_E}\frac{k_3}{k_1}\frac{\frac{K_E}{K_{a1}}k_1 + k_5}{1 + \frac{k_3}{k_{-1}}}$$

• S, P, B, and BH^+ apparent (Michaelis) binding constants

$$K_S = \frac{k_3}{k_1}\frac{1 + \frac{k_{-1}}{k_2[W]}}{1 + \frac{k_3}{k_2[W]}} \approx \frac{k_3}{k_1}; K_B = \frac{2}{k_4}\frac{k_3}{1 + \frac{k_3}{k_2[W]}} \approx \frac{2k_3}{k^\infty}\frac{K_E + K_{a2}}{K_E} = K_{B\infty}\frac{K_E + K_{a2}}{K_E}; K_P = 2\frac{K_{a1}}{K_E}\frac{k_3}{k_1}\frac{1 + \frac{k_{-1}}{k_2[W]}}{1 + \frac{k_3}{k_{-1}}} \approx \frac{2}{k_1\left(\frac{1}{k_3} + \frac{1}{k_{-1}}\right)}\frac{K_{a1}}{K_E}$$

$$K_{BH} = \frac{K_B}{4}\frac{K_P}{K_S}\frac{K_E}{K_{a1}}\frac{K_E}{K_{a2}} = \frac{1}{k_4}\frac{K_E}{K_{a2}}\left(\frac{1}{k_3} + \frac{1}{k_{-1}}\right)^{-1} \approx \frac{1}{k_\infty}\frac{K_E + K_{a2}}{K_{a2}}\left(\frac{1}{k_3} + \frac{1}{k_{-1}}\right)^{-1} = K_{BH\infty}\frac{K_E + K_{a2}}{K_{a2}}$$

• P apparent inhibition constants

$$K_{iP,1} = 2\frac{K_{a1}}{K_E}\left(\frac{k_1}{k_{-1}} + 2\frac{k_1}{k_3}\frac{k5}{\frac{K_E}{K_{a1}}k_1 + k_5\left(1 + \frac{k_{-1}}{k_2[W]}\right)}\right)^{-1} \approx 2\frac{K_{a1}}{K_E}\left(\frac{k_1}{k_{-1}} + 2\frac{k_1}{k_3}\frac{k5}{\frac{K_E}{K_{a1}}k_1 + k_5}\right)^{-1}; K_{iP,3} = \frac{K_{a1}}{K_E}\frac{k_3}{k_1}\frac{\frac{K_E}{K_{a1}}k_1 + k_5\left(1 + \frac{k_{-1}}{k_2[W]}\right)}{k5} \approx \frac{K_{a1}}{K_E}\frac{K_3}{k_1}\frac{\frac{K_E}{K_{a1}}k_1 + k_5}{k5}$$

$$K_{iP,2} = \frac{K_{a1}}{K_E}\frac{k_3}{k_1}\left(1 + \frac{k_{-1}}{k_2[W]}\right) \approx \frac{K_{a1}}{K_E}\frac{k_3}{k_1} = \frac{K_{a1}}{K_E}K_S; K_{K_{iP,5}} = \frac{K_{a1}}{K_E}\frac{k_{-1}}{k_1}\left(1 + \frac{k_3}{k_2[W]}\right) \approx \frac{K_{a1}}{K_E}\frac{k_{-1}}{k_1} = \frac{1}{2}\left(\frac{1}{K_{iP,1}} - \frac{1}{K_{iP,3}}\right)^{-1}$$

$$K_{iP,4} = \frac{K_{a1}}{K_E}\frac{k_{-1}}{k_1}\frac{k_3}{k_1}\frac{\frac{K_E}{K_{a1}}k_1 + k_5\left(1 + \frac{k_{-1}}{k_2[W]}\right)}{k_5}\left(\frac{k_1}{k_{-1}} + 2\frac{k_1}{k_3}\frac{k_5}{\frac{K_E}{K_{a1}}k_1 + k_5\left(1 + \frac{k_{-1}}{k_2[W]}\right)}\right) \approx \frac{K_{a1}}{K_E}\frac{k_{-1}}{k_1}\frac{k_3}{k_1}\frac{\frac{K_E}{K_{a1}}k_1 + k_5}{k_5}\left(\frac{k_1}{k_{-1}} + 2\frac{k_1}{k_3}\frac{k_5}{\frac{K_E}{K_{a1}}k_1 + k_5}\right) = \frac{{K_{iP,3}}^2}{K_{iP,3} - K_{iP,1}}$$

• Enzyme distribution equations

$$[E] \propto k_3k_4\left(1 + \frac{k_{-1}}{k_2[W]}\right)[B] + k_3\left(\frac{K_E}{K_{a1}}k_1 + k_5\left(1 + \frac{k_{-1}}{k_2[W]}\right)\right)[P] + \frac{K_E}{K_{a1}}k_1k_5[P]^2 + \frac{K_E}{K_{a1}}k_1k_4[B][P]$$

$$[ES] \propto k_1k_4\frac{k_3[S][B]}{k_2[W]} + k_3\frac{k_1}{k_{-1}}\left(\frac{K_E}{K_{a1}}k_1 + k_5\left(1 + \frac{k_{-1}}{k_2[W]}\right)\right)[S][P] + \frac{K_E}{K_{a1}}k_1k_5\frac{k_1}{k_{-1}}[S][P]^2 + \frac{K_{a2}}{K_{a1}}k_3k_4\frac{k_1}{k_{-1}}[BH^+][P] + \frac{K_E}{K_{a1}}k_1k_4\frac{k_1}{k_{-1}}[S][B][P]$$

$$[E_W] \propto \frac{K_{a1}}{K_E}k_3\left(\frac{K_E}{K_{a1}}k_1 + k_5\left(1 + \frac{k_{-1}}{k_2[W]}\right)\right)[S] + \frac{K_{a2}}{K_E}k_3k_4\left(1 + \frac{k_{-1}}{k_2[W]}\right)[BH^+] + k_1k_4[S][B] + k_1k_5[S][P]$$

$$[_HE] \propto \frac{K_{a1}}{K_E}k_3\left(\frac{K_E}{K_{a1}}k_1 + k_5\left(1 + \frac{k_{-1}}{k_2[W]}\right)\right)[S] + \frac{K_{a2}}{K_E}k_3k_4\left(1 + \frac{k_{-1}}{k_2[W]}\right)[BH^+] + \frac{K_{a2}}{K_{a1}}k_1k_4[P][BH^+] + k_1k_5[S][P]$$

Also, the following equalities can be straightforwardly established:

$$\kappa_{-1} = \kappa_1[\mathrm{W}]\frac{K_\mathrm{E}}{K_{\mathrm{a}1}} \tag{5}$$

$$k_{-2} = k_2[\mathrm{W}]\frac{k_1}{k_{-1}}\frac{K_\mathrm{E}}{K_{\mathrm{a}1}} \tag{6}$$

$$k_{-4} = k_4\frac{K_{\mathrm{a}1}}{K_\mathrm{E}} \tag{7}$$

$$[\mathrm{W}]k_{-5} = k_5\frac{K_{\mathrm{a}1}}{K_\mathrm{E}} \tag{8}$$

The S/P pair was viewed as any other proton donor/acceptor buffer. The forward intermolecular proton steps governed by the rate constants k_4 and k_5 were modeled according to a Brønsted relationship[12] for which a single asymptotic second-order intermolecular proton transfer rate constant, $k\infty$, was identified

$$k_4 = k_\infty\frac{K_\mathrm{E}}{K_\mathrm{E} + K_{\mathrm{a}2}} \tag{9}$$

$$k_5 = k_\infty\frac{K_\mathrm{E}}{K_\mathrm{E} + K_{\mathrm{a}1}} \tag{10}$$

The optimized rate constant aggregates with the inferred turnover and apparent Michaelis and inhibition constants along with their corresponding confidence intervals, are tabulated in Table 7. Parameter optimization was performed using the MatLab "nlinfit" routine. Ranking of the four kinetic model versions was carried out on the basis of AARE (Table 2) as defined by eq 1.

All four schemes led to reasonable values for the hydration turnover numbers (Table 7) in line with those published by Steiner et al.,[11] $(k_\mathrm{h})_{\max} = 10^6\ \mathrm{s}^{-1}$, and by Rowlett and Silverman,[12] $(k_\mathrm{h})_{\max} = 8 \times 10^5\ \mathrm{s}^{-1}$. Also, these schemes led to Michaelis constant for carbon dioxide, $K_\mathrm{S} \approx 8 \pm 2$ mM (Table 7), in very good agreement with the constants determined by Steiner et al.,[11] $K_\mathrm{S} = 8.3$ mM, and by Rowlett and Silverman,[12] $K_\mathrm{S} \approx 8 \pm 2$ mM.

The 3-parameter model (eq 11, Table 3) for the ordered Ter Bi Iso Ping Pong mechanism (scheme a) outperformed slightly the kinetic model of Rowlett and Silverman:[12] AARE = 18.6% on the whole data and 34.5% against the rate measurements with product inhibition only (Table 2). This mechanism implies that K_S and K_P are interrelated as $K_\mathrm{P} = 2\,(K_{\mathrm{a}1}/K_\mathrm{E})\,K_\mathrm{S}$ (Table 3). However, in spite of the fact that both K_S and K_P involve the same rate constants, κ_1 [W] and k_3, scheme a predicted an unacceptably large value for the bicarbonate Michaelis constant, $K_\mathrm{P} = 192 \pm 45$ mM (Table 7); in contrast with the accepted K_P values, an order of magnitude lesser.[11,15] This model was hence disqualified. Likewise, the 4-parameter model (eq 12, Table 4) for the random pseudo Quad Quad Iso Ping Pong (scheme b) was also dismissed for not being physical ($k_5 < 0$) in addition to the very large bicarbonate Michaelis constant, $K_\mathrm{P} = 222 \pm 77$ mM, it led to (Table 7).

The 4-parameter model given in eq 13, Table 5 for the ordered pseudo Ter Bi Iso Ping Pong mechanism (scheme c) led to a further reduction in global AARE (16.2%). Although scheme c seems more coherent than schemes a and b, it was not retained because it failed to account for the couplings between S and P as suggested by a lack of robustness to account for those hydration rates obtained under product inhibition (AARE inflation from 16.2% to 28%, Table 2).

Table 7. Optimized Rate Constant Aggregates and Inferred Turnover and Apparent Michaelis and Inhibition Constants

rate constant aggregates	turnover and apparent Michaelis and inhibition constants
scheme a	
$k_1[\mathrm{W}] = (1.4 \pm 0.2) \times 10^8\ \mathrm{s}^{-1}$	$k_\mathrm{h} = k_\mathrm{d} = (9.6 \pm 1.4) \times 10^5\ \mathrm{s}^{-1}$
$k_3 = (9.6 \pm 1.4) \times 10^5\ \mathrm{s}^{-1}$	$K_\mathrm{S} = (7.1 \pm 1.7)$ mM
$k_\infty = (3.3 \pm 0.5) \times 10^8\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$	$K_{\mathrm{B}\infty} = (5.9 \pm 1.2)$ mM; $K_{\mathrm{BH}\infty} = (2.9 \pm 0.6)$ mM
	$K_\mathrm{p} = (192 \pm 45)$ mM
scheme b	
$k_3\left(1 + \frac{K_{\mathrm{a}1}k_5}{K_\mathrm{E}k_1[\mathrm{W}]}\right) = (1.0 \pm 0.2) \times 10^6\ \mathrm{s}^{-1}$	$k_\mathrm{h} = k_\mathrm{d} = (1.0 \pm 0.2) \times 10^6\ \mathrm{s}^{-1}$
$\frac{k_3}{k_1[\mathrm{W}]} = (8.2 \pm 2.8) \times 10^{-3}$	$K_\mathrm{S} = (8.2 \pm 2.8)$ mM
$k_\infty = (3.3 \pm 0.4) \times 10^8\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$	$K_{\mathrm{B}\infty} = (6.4 \pm 1.4)$ mM; $K_{\mathrm{BH}\infty} = (3.2 \pm 0.7)$ mM
$k_5 < 0$	$K_\mathrm{P} = (222 \pm 77)$ mM
	$K_{iP,1} < 0$
scheme c	
$k_1 = (1.2 \pm 0.2) \times 10^8\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$	$k_\mathrm{h} = (1.1 \pm 0.2) \times 10^6\ \mathrm{s}^{-1}$; $k_\mathrm{d} = (3.8 \pm 1.2) \times 10^5\ \mathrm{s}^{-1}$
$k_{-1} = (5.7 \pm 2.7) \times 10^5\ \mathrm{s}^{-1}$	$K_\mathrm{s} = (9.7 \pm 2.3)$ mM
$k_3 = (1.1 \pm 0.2) \times 10^6\ \mathrm{s}^{-1}$	$K_{\mathrm{B}\infty} = (6.5 \pm 2.7)$ mM; $K_{\mathrm{BH}\infty} = (1.1 \pm 0.5)$ mM
$k_\infty = (3.5 \pm 0.4) \times 10^8\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$	$K_\mathrm{P} = (88.4 \pm 32)$ mM
	$K_{iP,1} = (133.3 \pm 67.6)$ mM
	$K_{iP,2} = (131.3 \pm 30.7)$ mM
	$K_{iP,5} = (66.6 \pm 33.8)$ mM
scheme d	
$\frac{k_3}{k_1} = (9.5 \pm 2.3) \times 10^{-3}$ M	$k_\mathrm{h} = (1.1 \pm 0.3) \times 10^6\ \mathrm{s}^{-1}$; $k_\mathrm{d} = (9.4 \pm 4.9) \times 10^4\ \mathrm{s}^{-1}$
$\frac{K_\mathrm{E}}{K_{\mathrm{a}1}}k_1 + k_5 = (8.4 \pm 1.1) \times 10^6\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$	$k_\mathrm{S} = (9.5 \pm 2.3)$ mM
$k_\infty = (4.2 \pm 0.5) \times 10^8\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$	$K_{\mathrm{B}\infty} = (5.2 \pm 1.5)$ mM; $K_{\mathrm{BH}\infty} = (0.23 \pm 0.12)$ mM
$\frac{k_1}{k_{-1}} = (11.1 \pm 4.5) \times 10^2\ \mathrm{M}^{-1}$	$K_\mathrm{P} = (22.2 \pm 8.3)$ mM
	$K_{iP,1} = (14.8 \pm 4.0)$ mM
	$K_{iP,2} = (127.9 \pm 30.9)$ mM
	$K_{iP,3} = (37.9 \pm 25.9)$ mM
	$K_{iP,4} = (61.9 \pm 44.1)$ mM; $K_{iP,5} = (12.2 \pm 7.0)$ mM

The 4-parameter pseudo random Quad Quad Iso Ping Pong mechanism with one transitory complex and fast solvation in the enzyme binding cavity was retained (scheme d, eq 14, Table 6). It exhibited by far the lowest overall error (AARE = 11.7%) and robust handling of the kinetic measurements carried out at [P] ≠ 0 (AARE = 13.2%, Table 2), beside it led to Michaelis constants for carbon dioxide, bicarbonate, and unprotonated buffer in agreement with the literature findings (Table 7). Also, the model adequacy to represent the reversible hydration rates of CO_2 by HCA II can be appreciated visually from the parity plot of Figure 1d.

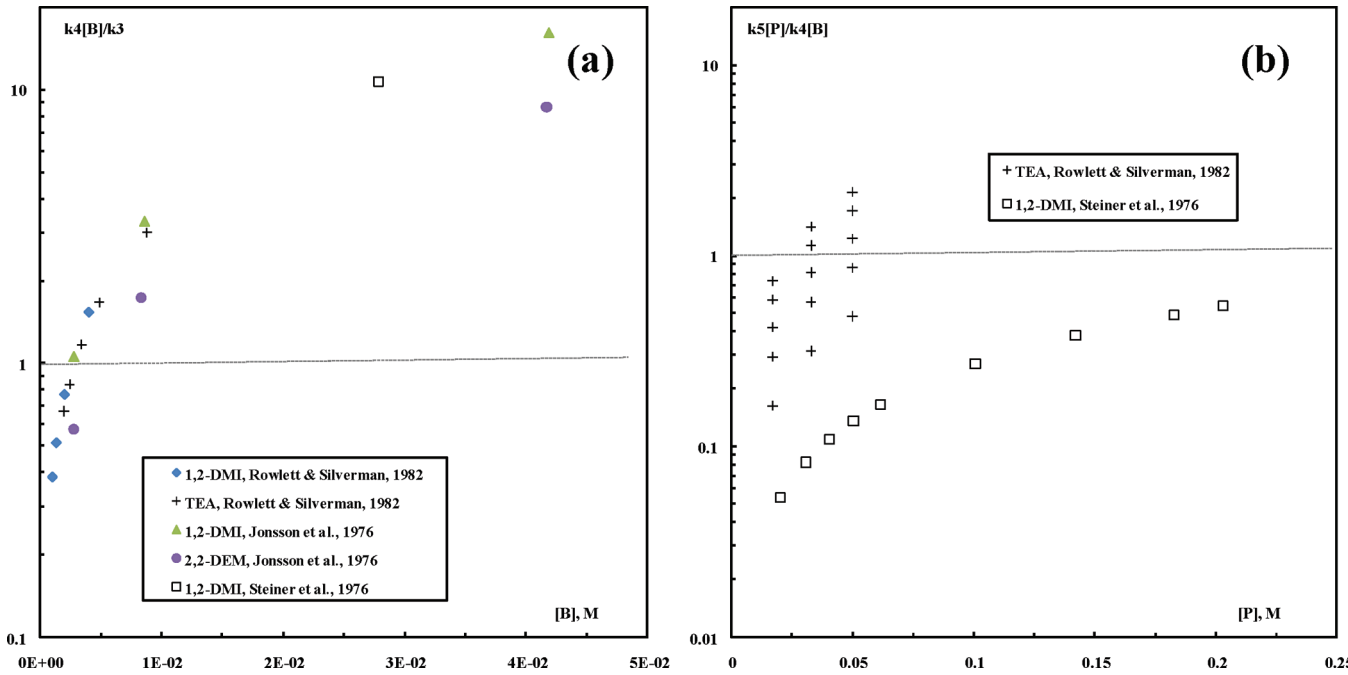

Figure 3. Time scale ratios of (a) intermolecular buffer and intramolecular proton transfers, (b) intermolecular buffer and bicarbonate proton transfers, scheme d. Data from Rowlett and Silverman,[12] Steiner et al.,[8] and Jonsson et al.[13]

The time scale ratios, $k_4[B]/k_3$, between the intermolecular buffer and the intramolecular proton transfer steps computed for scheme d kinetic model are shown in Figure 3a. They provide post facto confirmation that Steiner et al.[8] measurements were tinged nearly fully by intramolecular controlling step. Jonsson et al.[13] and Rowlett and Silverman[12] experiments sat astride from prevailing intermolecular control at low buffer concentration to intramolecular control at high buffer concentration. The low and high buffer concentration ranges are incorporated coherently using one single kinetic model (eq 14, Table 6) fulfilling the first question enunciated earlier.

To appraise the importance of possible competitive intermolecular proton transfer step by the CO_2/HCO_3^- pair with respect to the external buffer, the time scale ratios, $k_5[P]/k_4[B]$, were plotted in Figure 3b for the kinetic experiments in presence of product inhibition. The likelihood of this product-mediated proton transfer step is especially evident in the low-concentration buffer tests of Rowlett and Silverman[12] observing that $k_5[P] > k_4[B]$ for want of other proton donors due to an overwhelming bicarbonate concentration ($[P]/[B] = 26$). This competitive step can also contribute up to 60% of the buffer $k_4[B]$ rate constant for the high-concentration buffer tests of Steiner et al.[8] Hence, scheme d response to second question is affirmative with regard to contributing intermolecular proton transfer by the CO_2/HCO_3^- pair. The model suggests this role could be emphasized in product inhibition conditions at high [P]/[B] ratios, especially if buffer and CO_2 acid dissociation constants are close to each other.

The enzyme distribution equations in Table 6 reveal that $[S]\cdot[P]$ coupling terms arise in eq 14 if isomerization is conjectured along with a transitory complex ES: terms in $k_3[S]\cdot[P]$ and $k_4[S]\cdot[P]\cdot[B]$, and if bicarbonate also acts as proton acceptor: $k_5[S]\cdot[P]$ term in $[E_W]$ and $[_HE]$. The proton acceptor ability of P also gives rise to $[P]^2$ and $[S]\cdot[P]^2$ couplings via [E] and [ES], respectively.

Canceling the $[S]\cdot[P]$ coupling ($K_{iP,1} \rightarrow \infty$) in the pseudo random Quad Quad Iso Ping Pong mechanism inflated the AARE from 13.2% to 73.5% for the data at $[P] \neq 0$. Similarly, erasure of $[P]^2$ contribution ($K_{iP,3} \rightarrow \infty$) magnified AARE from 13.2% to 47% for the data at $[P] \neq 0$, whereas bringing to zero the $[S]\cdot[P]^2$ coupling ($K_{iP,4} \rightarrow \infty$) increased AARE from 13.2% to 26.4%. Finally, removing the coupling $[S]\cdot[P]\cdot[B]$ coupling HCO_3^- ($K_{iP,5} \rightarrow \infty$) rose the error from 13.2% to 52.4%. This sensitivity analysis revealed that the enzyme isomerization and intermolecular CO_2/HCO_3^--subtended proton transfer via $[CO_2]\cdot[HCO_3^-]$ coupling, the CO_2/HCO_3^--subtended proton transfer via $[CO_2]\cdot[HCO_3^-]^2$ couplings, and the enzyme−substrate transitory complex via $[CO_2]\cdot[HCO_3^-]\cdot[B]$ coupling, all mattered in the goodness of fit of scheme d kinetic model. The model performance to capture the effects because of inclusion of product inhibition in the hydration rate to highlight the above couplings can be glanced from Figure 4. This asserts affirmatively the third and last question enunciated in the introduction.

Conclusion

Four variants of the two-step Ping Pong mechanism for the reversible hydration of carbon dioxide to bicarbonate catalyzed by free human carbonic anhydrase II (HCA II) in solutions were derived and their goodness-of-fit to match measured initial hydration rates tested.

The pseudo (i.e., no central complex) ordered Ter Bi Iso Ping Pong mechanisms assumed three substrates (CO_2, water, unprotonated buffer) and two products (HCO_3^-, protonated buffer), alternatively accounting for one or no transitory complex. Likewise, four substrates (CO_2, water, unprotonated buffer, HCO_3^-) and as many products (HCO_3^-, protonated buffer, CO_2, and water), were involved in the pseudo random Quad Quad Iso Ping Pong mechanisms.

The pseudo random Quad Quad Iso Ping Pong mechanism with one transitory complex was retained in which competitive intermolecular proton transfer by the CO_2/HCO_3^- pair with respect to external buffer was implied. To allow consideration for simultaneous limiting intra- and intermolecular proton transfer steps inclusive of the contribution of CO_2/HCO_3^- pair, a databank for the CO_2 catalyzed hydration rates mediated by HCA II with and without HCO_3^- inhibition was constituted, embracing low and high concentrations of external buffers. The

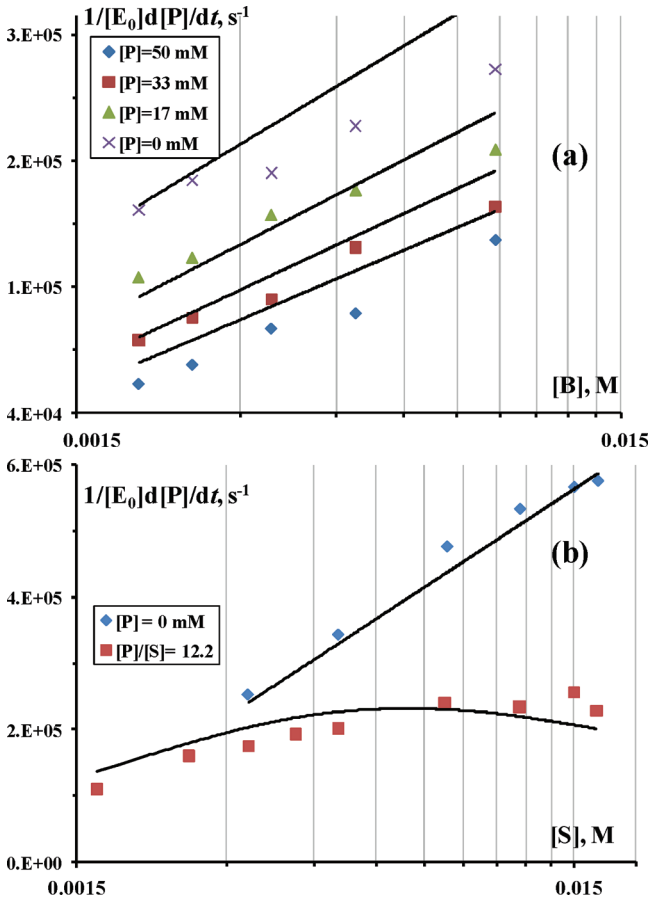

Figure 4. Scheme d model predictions of product inhibition on initial CO_2 hydration rate by human carbonic anhydrase II with couplings enzyme isomerization and S/P proton transfer ([S]·[P]); S/P proton transfer ($[P]^2$ and $[S]\cdot[P]^2$); ES transitory complex ([S]·[P]·[B]). Data from (a) Rowlett and Silverman[12] and (b) Steiner et al.[8] Solid lines calculated using Table 6.

4-parameter model yielded an average error of 11.7% ~8-fold lesser than current models with equal number of parameters. In addition to enzyme isomerization, the model was derived to account for CO_2/HCO_3^- proton transfer and enzyme−substrate transitory complex.

Supporting Information Available: Set of experimental kinetic data is provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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Received for review June 22, 2010
Revised manuscript received August 5, 2010
Accepted August 12, 2010

IE101338R