A model of carbon dioxide assimilation in Chlamydomonas reinhardii

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Keywords
Bicarbonate transport, Carbon dioxide concentrating system, Chlamydomonas, Chlorophyta, Mutant (Chlamydomonas), Photosynthesis (modeling)

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A model of carbon dioxide assimilation in *Chlamydomonas reinhardii*

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**Abstract.** A simple model of photosynthetic CO₂ assimilation in *Chlamydomonas* has been developed in order to evaluate whether a CO₂-concentrating system could explain the photosynthetic characteristics of this alga (high apparent affinity for CO₂, low photorespiration, little O₂ inhibition of photosynthesis, and low CO₂ compensation concentration). Similarly, the model was developed to evaluate whether the proposed defects in the CO₂-concentrating system of two *Chlamydomonas* mutants were consistent with their observed photosynthetic characteristics. The model treats a *Chlamydomonas* cell as a single compartment with two carbon inputs: passive diffusion of CO₂, and active transport of HCO₃⁻. Internal inorganic carbon was considered to have two potential fates: assimilation to fixed carbon via ribulose 1,5-bisphosphate carboxylase-oxygenase or exiting the cell by either passive CO₂ diffusion or reversal of HCO₃⁻ transport. Published values for kinetic parameters were used where possible. The model accurately reproduced the CO₂-response curves of photosynthesis for wild-type *Chlamydomonas*, the two mutants defective in the CO₂-concentrating system, and a double mutant constructed by crossing these two mutants. The model also predicts steady-state internal inorganic-carbon concentrations in reasonable agreement with measured values in all four cases. Carbon dioxide compensation concentrations for wild-type *Chlamydomonas* were accurately predicted by the model and those predicted for the mutants were in qualitative agreement with measured values. The model also allowed calculation of approximate energy costs of the CO₂-concentrating system. These calculations indicate that the system may be no more energy-costly than C₄ photosynthesis.

**Key words:** Bicarbonate transport – Carbon dioxide concentrating system – *Chlamydomonas* – Chlorophyta – Mutant (*Chlamydomonas*) – Photosynthesis (modeling).

**Introduction**

Unicellular green algae and cyanobacteria acclimate physiologically to the CO₂ concentration under which they are grown (Berry et al. 1976; Findenegg 1976; Hogetsu and Miyachi 1977; Lloyd et al. 1977; Badger et al. 1980; Kaplan et al. 1980). When compared with those grown at enriched (1–5%) CO₂ concentrations, algae grown at atmospheric CO₂ concentration (0.03–0.04%) have a much higher apparent affinity for CO₂ in photosynthesis, lower apparent photorespiration at atmospheric CO₂ levels, less O₂-inhibition of photosynthesis at atmospheric CO₂ levels, and lower CO₂ compensation concentrations. It may be possible to explain these characteristics based on a proposed CO₂-concentrating pathway in these microalgae (Badger et al. 1980; Kaplan et al. 1980). Energy-dependent inorganic-carbon accumulation has been demonstrated to occur to very high levels inside air-adapted but not CO₂-enriched *Chlamydomonas reinhardii* (Badger et al. 1980) and other algae (Kaplan et al. 1980; Beardall and Raven 1981; Zenvirth and Kaplan 1981), and a saturable transport component has been demonstrated for the inorganic-carbon accumulation of both *Anabaena* (Kaplan et al. 1980) and *Chlamydomonas* (Spalding and Ogren 1983).
The ability to accumulate inorganic carbon is inducible (Badger et al. 1980; Spalding and Ogren 1982), and the enzyme carbonic anhydrase (CA) is induced simultaneously. Carbonic anhydrase catalyzes the reversible hydration and dehydration of CO$_2$ and HCO$_3^-$, respectively. Based on studies with a CA-deficient mutant of C. reinhardii and with wild-type C. reinhardii using the CA inhibitor ethoxyxolamide, it has been suggested that an essential role of CA in Chlamydomonas photosynthesis is dehydration of HCO$_3^-$ transported into the cytoplasm in order to supply CO$_2$ for photosynthesis (Spalding et al. 1983a; Spalding and Ogren 1985). A second mutant of C. reinhardii, which has reduced capacity for inorganic-carbon accumulation and probably for inorganic-carbon transport, has also been isolated and characterized (Spalding et al. 1983b).

In this paper a model of carbon assimilation by Chlamydomonas is described. The model was developed in order to evaluate whether on active HCO$_3^-$-transport system could reasonably explain the photosynthetic characteristics of this alga. In addition, the model was used to evaluate whether the proposed defects of the two mutants mentioned above were consistent with their observed photosynthetic characteristics, assuming the active HCO$_3^-$-transport model to be correct.

**Materials and Methods**

Wild-type (strain 2137 mt +) and mutant Chlamydomonas reinhardii strains ca-1-12-1C mt+ (Spalding et al. 1983a), pmp-1-16-5K mt+ (Spalding et al. 1983b), and ca pmp mt+ (Spalding et al. 1983c) were grown in liquid, minimal medium as described previously (Spalding et al. 1983a). External CA was assayed by a previously described method (Spalding and Ogren 1982) using washed, intact cells rather than cell extracts (see also Spalding et al. 1985). Chlorophyll (Chl) was determined spectrophotometrically after extraction into 96% ethanol (Wintermans and De Mots 1965). Cells were counted in a hemocytometer after killing with I$_2$-KI solution.

Cell volume was estimated using silicon-oil-filtering centrifugation with the nonpermeable labeled solute [14C]sorbitol and $^{3}$H$_2$O by the method of Heldt (1980). Average internal pH was estimated by the distribution of the labeled weak acid [14C]formic acid following silicon-oil-filtering centrifugation, by the method of Heldt (1980). Internal inorganic-carbon concentration was estimated following silicon-oil-filtering centrifugation by a previously described method (Spalding and Ogren 1983).

The CO$_2$ compensation concentrations were measured by infrared gas analysis in a closed system as previously described (Spalding et al. 1983a).

Relative quantum efficiencies were estimated from the light response curves for photosynthetic O$_2$ evolution in the presence of a saturating concentration (5 mM) of NaHCO$_3$. Oxygen evolution was monitored as described previously (Spalding et al. 1983a). Light was supplied with a slide projector and modulated using Kodak neutral-density filters.

### Model of CO$_2$ assimilation

**Definition of terms used in model**

- $m_l$: unit of internal volumes
- $m_e$: unit of external volumes
- $C_i$: internal CO$_2$ concentration (nmol ml$^{-1}$)
- $B_i$: internal HCO$_3^-$ concentration (nmol ml$^{-1}$)
- $C_e$: external CO$_2$ concentration (nmol ml$^{-1}$)
- $B_e$: external HCO$_3^-$ concentration (nmol ml$^{-1}$)
- $K_d$: CO$_2$ permeability coefficient of limiting cell membrane ($5 \cdot 10^{-4}$ cm s$^{-1}$)
- $K_{do}$: O$_2$ permeability coefficient of limiting cell membrane ($6 \cdot 10^{-4}$ cm s$^{-1}$)
- $A$: area concentration of the cell surface ($15 \text{ cm}^2 \text{ ml}^{-1}$)
- $I_v$: concentration of cell internal volume in system ($1.5 \cdot 10^{-3}$ ml ml$^{-1}$)
- $T_v$: total volume of the system (1 ml$_v$

- $P$: Chl concentration of system (30 mg Chl ml$^{-1}$)
- $K_i$: Michaelis constant of transport in forward direction for HCO$_3^-$ (130 nmol ml$^{-1}$)
- $V_{f}$: $V_{max}$ for HCO$_3^-$ transport (0.35 nmol mg$^{-1}$ Chl s$^{-1}$ for wild-type; 0.0233 nmol mg$^{-1}$ Chl s$^{-1}$ for pmp-1 mutant and double mutant)
- $K_c$: Michaelis constant of transport in reverse direction for HCO$_3^-$ (33.250 nmol ml$^{-1}$)
- $K_{ba}$: Michaelis constant of CA for CO$_2$ (301.33 nmol ml$^{-1}$)
- $V_{ba}$: $V_{max}$ of CA for HCO$_3^-$ dehydation (41.2 nmol mg$^{-1}$ Chl s$^{-1}$)
- $C_f$: fixed carbon (nmol)
- $O_i$: internal O$_2$ concentration (nmol ml$^{-1}$)
- $K_e$: Michaelis constant of RuBPC/O for CO$_2$ (30 nmol ml$^{-1}$)
- $K_o$: inhibition constant ($K_i$) for O$_2$ of RuBPC (480 nmol ml$^{-1}$)
- $V_e$: apparent $V_{max}$ for RuBPC (0.055 nmol mg$^{-1}$ Chl s$^{-1}$)
- $O_e$: external O$_2$ concentration (nmol ml$^{-1}$)
- $v_1$: rate of dehydration of $B_i$ to $C_i$ catalyzed by CA (nmol s$^{-1}$)
- $v_2$: rate of hydration of $C_i$ to $B_i$ catalyzed by CA (nmol s$^{-1}$)
The model

The model was developed by treating a *Chlamydomonas* cell as a single, spherical compartment (Fig. 1) which exchanges inorganic carbon with its environment through bidirectional passive diffusion of CO$_2$ ($v_9$) and bidirectional transport of HCO$_3^-$ ($v_6$ and $v_7$). Internal inorganic carbon is assumed to undergo reversible hydration-dehydration of CO$_2$-HCO$_3^-$ (C$_i$-B$_i$) either uncatalyzed ($v_3$ and $v_4$) or catalyzed by CA ($v_1$ and $v_2$). For the sake of simplicity, external CO$_2$ (C$_e$) and HCO$_3^-$ (B$_e$) are assumed to be in continuous equilibrium. This assumption is based on the observation that *C. reinhardii* has abundant CA activity in the periplasmic space (Kimpel et al. 1983). The same is true for the mutants utilized in this work (Table 1; Spalding et al. 1985). Internal CO$_2$ (C$_i$) also serves as the substrate for photosynthetic CO$_2$ assimilation ($v_8$) resulting in the accumulation of fixed carbon (C$_f$).

It was necessary to estimate the internal volume and surface area of *C. reinhardii*. The cellular volume determined using a labeled nonpermeable solute was approx. 50 μl mg$^{-1}$ Chl (53 ± 13 μl mg$^{-1}$ Chl, n = 38). Using this volume and the experimentally determined value of approx. 1.5 μg Chl/10$^6$ cells (1.49 ± 13 μg Chl/10$^6$ cells, n = 7), cellular surface area (assuming spherical cells) can be calculated to be approx. 0.55 cm$^2$ μg$^{-1}$ Chl for *C. reinhardii*. For a cell-suspension Chl concentration (P) of 30 μg Chl ml$^{-1}$ the cell internal-volume concentration (I$_v$) is 1.5$^{-10^{-3}}$ ml ml$^{-1}$ and the cell surface-area concentration (A) is 15.5 cm$^2$ ml$^{-1}$. These values, if we make the further assumption of 1 ml total volume (T$_v$) for the whole system, allow all rates to be expressed in nmol s$^{-1}$ even though units of rate constants may be in volume or area units.

Net passive diffusion of CO$_2$ across the cell membrane is a function of the CO$_2$ permeability coefficient ($K_d$) of the limiting membrane (including the cell wall and the boundary layer on either side of the membrane), and the CO$_2$ concentration gradient from inside (C$_i$) to outside (C$_o$):

$$v_9 = K_d(C_i - C_o) A T_v.$$  
 eqn. 1

When the CO$_2$ concentration outside is higher than that inside (C$_e$ > C$_i$) net passive diffusion of CO$_2$ is from outside to inside and $v_9$ is negative.

Since the $K_d$ for *Chlamydomonas* is not known the value of this permeability coefficient was adjusted within certain limits to obtain a reasonable fit between the model and wild-type *Chlamydo-

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### Table 1. External carbonic-anhydrase activity in five strains of *Chlamydomonas reinhardii*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Units carbonic anhydrase mg$^{-1}$ Chl$^p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type (2137)</td>
<td>842 ± 69</td>
</tr>
<tr>
<td>ca-1-12-1 C</td>
<td>325 ± 101</td>
</tr>
<tr>
<td>prop-1-16-5 K</td>
<td>747 ± 57</td>
</tr>
<tr>
<td>ca pnp</td>
<td>333 ± 89</td>
</tr>
</tbody>
</table>

*a* Determined in cells grown with 5% CO$_2$ but transferred to air levels of CO$_2$ for 2 d. Means (± SD) of at least five determinations.
monas in terms of the photosynthetic CO₂ response curve. The upper limit for Kd was assumed to be that reported for erythrocyte plasma membranes, 8·10⁻³ cm s⁻¹ (Silverman et al. 1976), and the lower limit was assumed to be that estimated for the halophytic green alga Dunaliella salina, 10⁻⁵ cm s⁻¹ (Zenvirth and Kaplan 1981).

The HCO₃⁻ transport into the cell was assumed to exhibit simple Michaelis-Menten kinetics:

\[ v_e = \frac{V_i B_i P T_v}{(B_i + K_i)} \]  

The \( K_m \) for HCO₃⁻ (\( K_i \)) used was 130 μM as previously estimated from inorganic-carbon uptake kinetics in Chlamydomonas (Spalding and Ogren 1983). The \( V_{max} \) for transport (\( V_i \)) was adjusted to obtain a reasonable fit between the model and wild-type Chlamydomonas in terms of the photosynthetic CO₂-response curve, but did not differ much from the value (1400 μmol mg⁻¹ Chl h⁻¹ = 0.39 nmol g⁻¹ Chl s⁻¹) previously estimated (Spalding and Ogren 1983).

Reason dictates that there must be some effect of accumulating HCO₃⁻ on the net rate of transport to limit the accumulation. Although there is no unequivocal evidence that HCO₃⁻ transport is reversible, an apparent reversal has been observed in CO₂-exchange studies (Spalding and Ogren 1985). This factor was incorporated into the model by assuming the transport to be reversible with equivalent \( V_{max} \) values in both directions and a \( K_m \) value for efflux (\( K_i \)) 250-fold higher (33250 μM) than that of influx to limit the ratio of internal to external HCO₃⁻ to 250, the approximate ratio observed in a CA-deficient mutant of C. reinhardii (Spalding et al. 1983a). Other approaches to resolving this problem (i.e. same \( K_m \)’s but different \( V_{max} \)’s or an energetic limitation on the internal to external HCO₃⁻ ratio) would be expected to yield similar results. Based on these considerations, HCO₃⁻ transport out of the cell is expressed as:

\[ v_7 = \frac{V_i B_i P T_v}{(B_i + K_i)} \]  

The rate of uncatalyzed dehydration of internal HCO₃⁻ at a constant internal pH is a function of the internal HCO₃⁻ concentration (\( B_i \)) and the overall rate constant (\( k_3 \)) for dehydration of HCO₃⁻ at the given pH (see Appendix):

\[ v_3 = k_3 B_i I_v T_v \]  

In a similar manner, uncatalyzed hydration of internal CO₂ is a function of the internal CO₂ concentration (\( C_i \)) and the overall rate constant (\( k_4 \)) for hydration of CO₂ at the given pH (see Appendix):

\[ v_4 = k_4 C_i I_v T_v \]  

Using the method of Heldt (1980) for estimation of internal pH by distribution of a labeled weak acid, the average internal pH of C. reinhardii in the light was found to be approx. 7.5 (7.48 ± 0.08, \( n = 13 \)), and this value was used as the internal pH in this model. At this pH, \( k_3 \) was calculated to be 2.94·10⁻³ s⁻¹ and \( k_4 \) to be 3.97·10⁻² s⁻¹ (see Appendix).

The interconversion of CO₂ and HCO₃⁻ catalyzed by CA may be described by using the rate for a single-substrate reaction which can be separated into the forward and reverse reaction. Therefore, the rate of dehydration of HCO₃⁻ is:

\[ v_1 = \frac{V_{ba} K_{ca} B_i}{K_{ba} K_{ca} + K_{ca} B_i + K_{ba} C_i} \]  

and the rate of hydration of CO₂ is:

\[ v_2 = \frac{V_{ca} K_{ca} C_i}{K_{ba} K_{ca} + K_{ca} B_i + K_{ba} C_i} \]  

where \( V_{ba} \) and \( V_{ca} \) are the maximal velocities of dehydration and hydration, respectively, while \( K_{ba} \) and \( K_{ca} \) are the Michaelis constants of the enzyme for HCO₃⁻ and CO₂, respectively.

The Michaelis constants and rate constants are not available for Chlamydomonas CA but probably do not differ too much from those that have been reported for the spinach enzyme (Pocker and Ng 1973; Pocker and Miksch 1978). The values for spinach are: \( K_{ca} = 1800 \) nmol ml⁻¹; \( K_{ba} = 3400 \) nmol ml⁻¹. The specific activity of Chlamydomonas CA in Wilbur-Anderson units was reported by Bundy and Cote (1980). This value, plus an estimate from Spalding and Ogren (1982) of the total CA activity in Chlamydomonas, allowed conversion of the rate constants to a Chl basis. The resultant estimates were 332 and 412 nmol g⁻¹ Chl s⁻¹ for \( V_{ca} \) and \( V_{ba} \), respectively. When these data were used our preliminary calculations indicated that the rates of interconversion of CO₂ and HCO₃⁻ inside the cell would be in great excess of all the other rates, indicating complete equilibration between these species. However, most of the CA activity appears to be external to the cells (Kimpel et al. 1983), so we reduced \( V_{ca} \) and \( V_{ba} \) by 90% (\( V_{ca} = 33.2 \) and \( V_{ba} = 41.2 \) nmol g⁻¹ Chl s⁻¹). Under these conditions, the model still predicted a complete equilibration.
between the internal CO₂ and HCO₃⁻ concentrations for wild-type *Chlamydomonas*. Finally, a slight adjustment of the rate constants and Michaelis constants reported for the spinach enzyme was necessary to make the equilibrium constant of the reaction catalyzed by CA (as calculated from the Haldane equation) equal to that used for the uncatalyzed reaction. This was accomplished by adjusting $K_{oa}$ to 30133 nmol ml⁻¹ (see Appendix).

The rate of photosynthetic assimilation of internal CO₂ ($v_8$) into fixed carbon ($C_f$) is determined by the kinetics of the enzyme ribulose 1,5-bisphosphate carboxylase-oxygenase (RuBPC/O) (Laing et al. 1974) and is a function of the internal CO₂ concentration ($C_i$)

$$v_8 = \frac{V_x C_i P_{T_x}}{C_i + K_c (1 + O_i/K_o)}$$  \hspace{1cm} \text{eqn. 8}

In this expression, $K_c$ and $K_o$ represent the $K_m$ for CO₂ and the $K_i$ for O₂, respectively, and the values for each are: $K_c = 30$ µM, $K_o = 480$ µM (Jordan and Ogren 1981). $V_x$ is the $V_{max}$ for RuBPC/O, but for the sake of simplicity the approximate maximum photosynthetic rate of the algae (200 µmol CO₂ mg⁻¹ Chl h⁻¹ = 0.055 nmol CO₂ gg⁻¹ Chl s⁻¹) was used instead. The RuBP concentration was assumed to be saturating in all cases.

Using the equations described above ($v_4 - v_{10}$), the differential rate equations for $C_i$ and $B_i$ were obtained. Approximate solutions of the changes in $C_i$ and $B_i$ with respect to time were obtained on a computer by using the Euler method (Savageau 1976, pp. 132–136). The time step utilized for iteration (2·10⁻⁴ s over first s, then 1·10⁻³ s thereafter) was selected as a reasonable compromise between computing time and accuracy. The data from the CO₂ time courses were then utilized to generate the photosynthetic CO₂-response curves as indicated in the figures.

**Results and discussion**

It was possible to obtain values from the literature for all kinetic parameters used in the model except the CO₂ permeability coefficient ($K_d$) for the limiting cell membrane and the $V_{max}$ for HCO₃⁻ transport ($V_t$). Even though the apparent $K_m$ for HCO₃⁻ transport (130 µM) of Spalding and Ogren (1983) was only a rough approximation, as the only available estimate it was used as the $K_c$ for the model. An estimate for $V_t$ was also given by Spalding and Ogren (1400 µmol mg⁻¹ Chl h⁻¹ = 0.39 nmol µg⁻¹ Chl s⁻¹) and was used as a guideline in construction of the model.

The value of $K_d$ is the kinetic parameter in which there is perhaps the greatest uncertainty. Biological membranes are considered to be relatively permeable to CO₂, with estimated permeability coefficients approximating 10⁻² cm s⁻¹ (Nobel 1974). An experimental estimate of the permeability coefficient of the erythrocyte membrane indicated a value of approx 8·10⁻³ cm s⁻¹ (Silverman et al. 1976), very close to the range suggested by Nobel. In contrast, Zervirth and Kaplan (1981) have estimated the permeability coefficient for CO₂ of the limiting membrane of the green alga *Dunaliella salina* to be 10⁻⁴–10⁻⁵ cm s⁻¹.

With other kinetic parameters set to published values, $K_d$ and $V_t$ were, within limits, varied independently to simulate the CO₂-response curve of photosynthesis for wild-type *C. reinhardii*. It was determined that reasonable fits could be obtained over a range of $K_d$ values from 5·10⁻³ to 5·10⁻⁵ cm⁻¹ s⁻¹ by adjusting $V_t$. By setting $K_d$ at 5·10⁻⁴ cm s⁻¹, a $V_t$ value (0.35 nmol µg⁻¹ Chl s⁻¹) was obtained which was similar to that estimated previously (Spalding and Ogren 1983) and similar to that determined for the cyanobacterium *Anabaena variabilis* (Kaplan et al. 1980). The CO₂-response curve using these values is illustrated in Fig. 2 along with points indicating the actual CO₂ response of photosynthesis in wild-type *C. reinhardii*.

The results illustrated in Fig. 2 demonstrate that an active HCO₃⁻-transport system could explain the high apparent affinity for CO₂ reported for air-adapted, wild-type-cells of *Chlamydomonas*. Internal inorganic-carbon concentrations predicted by the model were also in close agreement with those actually measured in wild-type *C. reinhardii* (Table 2).

The tolerance of the model to minor changes in $V_t$ and $K_d$ is relatively wide. A change of 10% in either value alone results in less than a 7% change in the predicted internal CO₂ concentration ($C_i$) when external CO₂ ($C_e$) is equivalent to normal air. Also, as was mentioned above, one can vary $K_d$ over quite a wide range yet still find a relatively reasonable value of $V_t$ for each $K_d$ value (Table 4) that allows a reasonable fit of the predicted to real CO₂-response curve.

A mutant of *C. reinhardii* deficient in CA activity (ca-1-12-1C) has been isolated and described (Spalding et al. 1983a). The deficiency appears to be only in internal CA activity (Spalding et al. 1985). This mutant accumulated internal inorganic carbon to a concentration much higher than the wild-type, yet was apparently CO₂-limited at air levels of CO₂. It was concluded that the deficiency...
Fig. 2. Comparison of an actual photosynthetic CO$_2$-response curve (open circles) of wild-type *Chlamydomonas reinhardii* with that predicted by the model (solid line). The actual CO$_2$-response curve was determined as described previously (Spalding et al. 1983a). Rates predicted by the model were steady-state rates at constant external inorganic-carbon concentrations of CA in the *ca-1* mutant resulted in accumulation of transported HCO$_3^-$ which only slowly underwent dehydration to CO$_2$. We initially questioned whether, at the very high internal inorganic-carbon concentrations (10–13 mM) observed at air levels of CO$_2$ in the *ca-1* mutant, HCO$_3^-$ would not still undergo uncatalyzed dehydration to CO$_2$ at a rate rapid enough to support a high photosynthetic rate. Preliminary calculations based on rate constant $k_3$, indicated that the rate of CO$_2$ supply from uncatalyzed dehydration of a 13 mM internal HCO$_3^-$ pool would only be approx. 7 μmol CO$_2$ mg$^{-1}$ Chl h$^{-1}$, compared with a photosynthetic rate in wild type of 150–180 μmol CO$_2$ mg$^{-1}$ Chl h$^{-1}$. The measured rate of photosynthesis in the *ca-1* mutant at air levels of CO$_2$ is 15–25 μmol CO$_2$ mg$^{-1}$ Chl h$^{-1}$, or 2–3 times higher than the predicted CO$_2$ supply rate. To further pursue this point, data were generated with the model assuming no internal CA activity. As can be seen from Fig. 3a, the CO$_2$-response curve of photosynthesis predicted by the model in the absence of internal

### Table 2. Comparison between measured and predicted internal inorganic carbon concentrations for wild-type and mutant strains of *C. reinhardii*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Initial external TIC (mM)</th>
<th>Measured internal TIC $^a$ (mM)</th>
<th>Model internal TIC $^e$ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>0.02</td>
<td>0.6–1.5$^a$</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>2–3.5$^b, c, d$</td>
<td>2.7</td>
</tr>
<tr>
<td><em>ca-1</em></td>
<td>0.02</td>
<td>2–5$^a$</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>10–13$^b, d$</td>
<td>15.5</td>
</tr>
<tr>
<td><em>pmp-1</em></td>
<td>0.08</td>
<td>0.3–0.5$^c, d$</td>
<td>0.2</td>
</tr>
<tr>
<td><em>ca pmp</em></td>
<td>0.08</td>
<td>3–5$^d$</td>
<td>9.0</td>
</tr>
</tbody>
</table>

$^a$ TIC = total inorganic carbon. Measured as maximum during a 120-s time course at pH 7.0 as in Spalding et al. (1983a)

$^b$ Spalding et al. 1983a. Maximum during a 120-s time course

$^c$ Spalding et al. 1983b. Maximum during a 120-s time course

$^d$ Spalding et al. 1983c. Maximum during a 120-s time course

$^e$ Calculated as maximum over 120 s at constant external inorganic-carbon concentration as indicated (pH 7.0)

Fig. 3A–C. Comparison of actual photosynthetic CO$_2$-response curves (open circles) of the *ca-1* mutant (A), the *pmp-1* mutant (B) and the *ca pmp* double mutant (C) of *Chlamydomonas reinhardii* with those predicted for each by the model (solid lines). The actual CO$_2$-response curves were determined as described previously (Spalding et al. 1983a). Rates predicted by the model were steady-state rates at constant external inorganic-carbon concentrations.
CA activity agreed well with the CO₂ response of the ca-1 mutant. In addition, the predicted internal inorganic-carbon concentrations were in close agreement with the measured values for the ca-1 mutant (Table 2). We conclude from this that the proposed defect in the ca-1 mutant is, within the framework of our model of the *Chlamydomonas* CO₂-concentrating pathway, consistent with the observed photosynthetic characteristics of this mutant.

A comparison of the rates predicted by the model of CO₂ diffusion (v₉) and of HCO₃⁻ dehydration (v₆) during steady-state photosynthesis in the ca-1 mutant indicated that CO₂ was diffusing into the cell (i.e. Cᵢ < Cₑ) at a rate approximately equal to the rate at which Bₑ was undergoing dehydration to Cᵢ. Thus a large part of the CO₂ fixed by ca-1 probably diffuses into the cell as CO₂ rather than being transported in as HCO₃⁻.

Another mutant of *C. reinhardii* (pmp-1-16-5 K) has been described which does not accumulate inorganic carbon to any substantial extent (Spalding et al. 1983b). This pmp-1 mutant was concluded to be deficient in, but not totally lacking, HCO₃⁻ transport. Because of the absence of any kinetic data on the HCO₃⁻ transport in this mutant, we decreased Vᵣ in the model until a reasonable fit to the actual CO₂-response curve of photosynthesis was obtained. The best fit was obtained using a Vᵣ value of 0.023 nmol μg⁻¹ Chl s⁻¹ (Fig. 3b). The internal inorganic-carbon concentration predicted by the model was slightly lower than the observed values for the pmp-1 mutant (Table 2). The modeling data do indicate that the photosynthetic characteristics of the pmp-1 mutant are consistent with a reduced capacity for HCO₃⁻ transport.

A double mutant (ca pmp) was constructed which contains both the ca-1 and the pmp-1 mutations (Spalding et al. 1983c). Since the model was able to predict reasonably well the photosynthetic characteristics of each mutant alone, it was of interest to test its accuracy at predicting these characteristics in an alga with both defects. The low Vᵣ (0.023 nmol μg⁻¹ Chl s⁻¹) of the pmp-1 model was combined with the absence of CA activity as in the ca-1 model to yield a model of carbon assimilation in ca pmp. The resultant calculated CO₂-response curve is illustrated in Fig. 3c along with data indicating the actual photosynthetic CO₂ response of the double mutant. The predicted CO₂-response curve slightly underestimates the actual data but, considering the simplicity of the model, the fit is reasonable. The internal inorganic-carbon concentration predicted for ca pmp by the model, although qualitatively similar, is somewhat higher than that actually measured (Table 2). As with the ca-1 mutant, analysis of the rates v₉ and v₃ indicated that the model predicts net diffusion of CO₂ into the cell as a major source of Cᵢ and thus Cᵢ'.

Air-adapted wild-type *Chlamydomonas* has a very low and relatively O₂-insensitive CO₂ compensation concentration (Spalding et al. 1983a). This characteristic can also be used to test whether the model is able to predict accurately the photosynthetic characteristic of *Chlamydomonas*. If one assumes that one photorespiratory CO₂ is released for each two phosphoglycolate molecules produced by RuBP oxygenase, a metabolic CO₂ compensation concentration can be predicted based on the kinetics of RuBPC/0. From Jordan and Ogren (1981) we obtain the relationship:

\[
v_c/v_o = (V_cK_0/V_oK_C) (C_i/O_i)
\]

where vₙ and vₙ are the velocities of carboxylation and oxygenation, respectively, Vₙ and Vₙ the maximal velocities of the two reactions, and Kₙ and Kₙ the Michaelis constants for CO₂ and O₂, respectively. The term \((V_cK_0/V_oK_C)\), called the substrate specificity factor by Jordan and Ogren, was determined to have a value of 61 for *C. reinhardii* (Jordan and Ogren 1981). Assuming one photorespiratory CO₂ molecule released for every two phosphoglycolate molecules formed, at the CO₂ compensation concentration \(v_c/v_o\) must equal 0.5. After inserting these values for \(v_c/v_o\) and \((V_cK_0/V_oK_C)\) the relationships becomes:

\[
C_i = 0.0082 \text{ O}_i.
\]

The metabolic or internal CO₂ compensation concentration can therefore be predicted for any given O₂ concentration (Table 3). At the CO₂ compensation concentration, net influx of inorganic carbon into the cell is zero. Thus HCO₃⁻ transport (v₆) must balance CO₂ diffusion out of the cell (v₉). The reverse reaction of the transporter (v₉) was assumed to be negligible compared with v₆ for these calculations. Assuming that Bₑ and Cᵢ as well as Bᵢ and Cₑ are in equilibrium and using values of Cᵢ calculated for internal CO₂ compensation concentrations it was possible to calculate the expected external CO₂ compensation concentration at a given O₂ concentration by setting v₆ equal to v₉ and solving for Cₑ.

\[
(C_i - C_e) K_d = \frac{V_c B_e P T_s}{(B_e + K_i)}
\]

If one assumes that Bₑ (1 nmol ml⁻¹) is negligible compared to Kᵢ (130 nmol ml⁻¹) and substitutes...
Table 3. Comparison of measured and predicted CO₂ compensation concentrations in C. reinhardtii

<table>
<thead>
<tr>
<th>O₂ concentration</th>
<th>Predicted internal CO₂ concentration</th>
<th>CO₂ compensation concentrations (μl-l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild-type</td>
<td>pmp-1</td>
</tr>
<tr>
<td></td>
<td>Measured</td>
<td>Model</td>
</tr>
<tr>
<td>%</td>
<td>μM</td>
<td>μM</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>0.2</td>
</tr>
<tr>
<td>21</td>
<td>265</td>
<td>2.2</td>
</tr>
<tr>
<td>50</td>
<td>631</td>
<td>5.0</td>
</tr>
</tbody>
</table>

References:

4.3 Cₑ for Bₑ (ratio of Bₑ to Cₑ at equilibrium at pH 7.0), then the expression solved for Cₑ becomes:

\[ Cₑ = C₁ \left( \frac{4.3 V₁ P}{Kₐ A Kᵣ} + 1 \right)^{-1} \]  

Since \( V₁, P, Kₐ, A, \) and \( Kᵣ \) are all constant values and the value of \( C₁ \) can be calculated as a function of the O₂ concentration (see above), it is possible to predict the CO₂ compensation concentration for both wild-type and the pmp-1 mutant using appropriate values of \( V₁ \) for each (Table 3). The CO₂ compensation concentration values predicted for wild-type *Chlamydomonas* agree with measured values. For the pmp-1 mutant the predicted values agree with those measured at 2% and 21% O₂ but are substantially lower at 50% O₂. There is some question as to whether in microalgae glycolate resulting from RuBP oxygenase activity leads to CO₂ release from glycine as in higher plants or whether some proportion of the glycolate is simply excreted. Although it is unclear what proportion of the glycolate formed is excreted, it has been demonstrated that excretion increases with increasing O₂ concentration (Berry et al. 1976). It is apparent that the assumption of one CO₂ released for every two phosphoglycolate molecules formed (see above) would lead to an overestimate of the CO₂ compensation concentration when glycolate is excreted, and that the overestimation is likely to be greater at high O₂ concentrations. This may, therefore, explain the substantial overestimation by the model of the CO₂ compensation concentration for the pmp-1 mutant at 50% O₂. Such an overestimation for wild-type *Chlamydomonas* might not be easily detected since the predicted compensation concentration is nearly at the lower level of detection even at 50% O₂.

Since the ca-1 mutant and the double mutant are both deficient in CA activity, the assumption of equilibration between Bᵢ and Cᵢ cannot be made. Thus the CO₂ compensation concentrations for these two mutants must be calculated in a different manner. In the absence of internal CA activity the rate of dehydration of Bᵢ to Cᵢ (v₃) can be assumed to be limiting in the sequence Bₑ v₀ Bᵢ v₃ Cᵢ. Thus, at the CO₂ compensation concentration the net rate of HCO⁻⁻ transport is determined by and equal to the HCO⁻⁻ dehydration rate. The rate of hydration of Cᵢ to Bᵢ can be assumed to be negligible under these conditions. Therefore, in the CA-deficient mutants it should be possible to predict the CO₂ compensation concentration by setting v₃ (= v₀) equal to v₄ and solving for Cₑ:

\[ (Cᵢ - Cₑ) Kᵣ A Tᵣ = Bₖ k₃ I₄ Tᵣ \]  

Substituting 250 Bₑ for Bᵢ (transport near equilibrium) and 4.3 Cₑ for Bₑ (thus Bₑ=1075 Cₑ) the expression solved for Cₑ becomes:

\[ Cₑ = C₁ \left( \frac{1075 k₃ I₄}{Kₐ A} + 1 \right)^{-1} \]
Since \( k_3, I, K_d, \) and \( A \) are all constant values, and \( C_i \) can be calculated as a function of the \( O_2 \) concentration (see above), it is possible to estimate the \( CO_2 \) compensation concentration of both the \( ca-1 \) mutant and the double mutant (Table 2). All parameters used for the calculation are identical for these two mutants, so the predicted compensation points are also expected to be identical. This part of the prediction is borne out since the measured \( CO_2 \) compensation concentrations of the \( ca-1 \) mutant and the double mutant are very similar at each \( O_2 \) concentration. Qualitatively the predicted compensation points match the measured compensation points in that they are quite high relative to the wild type and are \( O_2 \)-sensitive. Quantitatively, however, the predicted compensation points do not match well except at 21% \( O_2 \).

There is no obvious explanation for the very high \( CO_2 \) compensation concentrations observed in these mutants at 50% \( O_2 \), so it is difficult to speculate as to why the predictions of the model match so poorly in this instance. The measured compensation points at 50% \( O_2 \) are higher than the predicted internal or metabolic \( CO_2 \) compensation concentration (150 \( \mu \)M). This is also true of those measured at 2% \( O_2 \) and to a lesser extent at 21% \( O_2 \). It is possible that the unusually high \( CO_2 \) compensation concentrations observed in the \( ca-1 \) and \( ca \, pmp \) mutants could result, at least in part, from "dark-respiratory" (Krebs-cycle) \( CO_2 \) release. Lacking any reasonable estimate of the extent of this type of respiration in the light, however, we did not incorporate this factor into the model.

Apparent there is something we do not completely understand either about photorespiratory \( CO_2 \) release in \textit{Chlamydomonas}, or about the consequences of a deficiency of CA activity. It is of interest to note that the \( CO_2 \) compensation concentrations of wild-type \textit{Chlamydomonas} in the presence of a specific inhibitor of CA, ethoxyzolamide, are qualitatively similar to those of the \( ca-1 \) mutant (Spalding et al. 1983a) but, except at 2% \( O_2 \) are quantitatively very similar to those predicted by the model (30 \( \mu \)M at 2% \( O_2 \); 45 \( \mu \)M at 21% \( O_2 \); 87 \( \mu \)M at 50% \( O_2 \)).

If the resistance of the limiting cell membrane of \textit{Chlamydomonas} is large enough to allow retention of a large \( CO_2 \) gradient it also must result in an increase in the internal \( O_2 \) concentration \( (O_i) \), since \( O_2 \) is being produced inside the cell during photosynthesis. The extent of this \( O_2 \) accumulation and its effect on the photosynthetic rate in the model can be estimated. At steady-state photosynthesis the rate of \( O_2 \) diffusion out of the cell \( (v_{o10}) \) is equal to the rate of \( O_2 \) production \( (v_8) \). Since diffusion is inversely proportional to approx. the square root of the molecular weight of the diffusing species, the permeability coefficient for \( O_2 \) \( (K_{do}) \) can be estimated as:

\[
K_{do} = K_d \sqrt{\frac{\text{molecular weight } CO_2}{\text{molecular weight } O_2}} \\
= 6 \cdot 10^{-4} \text{ cm s}^{-1} \text{ eqn. 15}
\]

and the expression for \( V_{o1} \) expressed as:

\[
v_{10} = (O_i - O_o) K_{do} A T_v \text{ eqn. 16}
\]

Setting \( v_{10} \) equal to \( v_8 \) and rearranging we obtain an expression for estimating \( O_i \):

\[
O_i = O_o + \frac{v_8}{(K_{do} A T_v)} \text{ eqn. 17}
\]

At one-half the maximum photosynthetic rate the estimated internal \( O_2 \) concentration would rise from 256 \( \mu \)M to 348 \( \mu \)M. Because of the elevated internal \( CO_2 \) concentrations this would, however, reduce the photosynthetic rate by only about 5%. Therefore, as is the situation with \( C_4 \) plants (Berry and Farquhar 1978) and with CAM plants (Spalding et al. 1979), the characteristics of \textit{Chlamydomonas} which allow it to maintain a high intracellular \( CO_2 \) concentration also result in an accumulation of \( O_2 \), but the increased \( O_2 \) concentration has little effect on the photosynthetic rate because of the elevated \( CO_2/O_2 \) ratio.

An important question regarding the \( CO_2 \)-concentrating system of \textit{Chlamydomonas} and other unicellular algae is how energetically costly it is to operate. The inorganic-carbon transport apparently requires energy, probably as ATP (Ogawa et al. 1984). If one assumes that one molecule of ATP is required for transport of each \( HCO_3^- \) molecule the overall ATP requirement for assimilation and fixation of one molecule of \( CO_2 \) is at least four (three ATP required for reduction of \( CO_2 \) in the Calvin cycle). Any leakage of the transported inorganic carbon back out of the cell \( (CO_2 \) diffusion) would increase the ATP requirement. This "leak rate" is therefore quite important in evaluating the overall energy requirement for the \( CO_2 \)-concentrating system. Although this leak rate has never been measured, one can use the model to make some predictions about energy costs by comparing the rate of \( HCO_3^- \) transport into the cell \( (v_e) \) with the rate of \( CO_2 \) fixation \( (v_8) \). The ratio \( v_e/v_8 \) indicates how many \( HCO_3^- \) molecules must be transported for each \( CO_2 \) fixed and, thus, how many ATP's must be used for transport of each \( CO_2 \) fixed (assuming 1 ATP/\( HCO_3^- \) in transport).
Table 4. Predicted ATP requirement for CO₂-concentrating system of *C. reinhardii*

<table>
<thead>
<tr>
<th>$K_d$ (cm s⁻¹)</th>
<th>$V_1$ (nmol s⁻¹)</th>
<th>$v_8$ (nmol s⁻¹)</th>
<th>$v_6$ (nmol s⁻¹)</th>
<th>$v_9$ (nmol s⁻¹)</th>
<th>$v_6/v_8$</th>
<th>ATP required (per CO₂ fixed)</th>
<th>Total ATP requirement (per CO₂ fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5·10⁻³</td>
<td>52.5</td>
<td>1.2</td>
<td>9.7</td>
<td>8.5</td>
<td>8.1</td>
<td>8.1</td>
<td>11.1</td>
</tr>
<tr>
<td>10⁻³</td>
<td>15.0</td>
<td>1.2</td>
<td>2.9</td>
<td>1.7</td>
<td>2.4</td>
<td>2.4</td>
<td>5.4</td>
</tr>
<tr>
<td>5·10⁻⁴</td>
<td>10.5</td>
<td>1.2</td>
<td>2.0</td>
<td>0.8</td>
<td>1.7</td>
<td>1.7</td>
<td>4.7</td>
</tr>
<tr>
<td>10⁻⁴</td>
<td>7.5</td>
<td>1.2</td>
<td>1.4</td>
<td>0.2</td>
<td>1.2</td>
<td>1.2</td>
<td>4.2</td>
</tr>
</tbody>
</table>

The difference between $v_6$ and $v_8$ is equal to the CO₂ leak rate ($v_9$). Table 4 lists calculated values for these parameters at an external CO₂ concentration (10 nmol ml⁻¹) equivalent to that of normal air. Since the resistance to CO₂ diffusion ($K_d$) of the limiting membrane is important in determining the CO₂ leak rate, and since the $K_d$ used in the model was chosen somewhat arbitrarily, the calculations for energy requirement in Table 4 have been performed with a range of $K_d$ values. The HCO₃⁻ transport $V_{max}$ ($V_1$) was adjusted to give equivalent CO₂ fixation rates (equivalent internal CO₂ concentrations) for each $K_d$ value.

With a $K_d$ value of 5·10⁻³ cm s⁻¹, which is roughly equivalent to that estimated for erythrocyte membranes, the leak rate ($v_9$) would be sevenfold higher than the CO₂-fixation rate ($v_8$) and the predicted ATP requirement (11.1 ATP/CO₂ fixed) would be prohibitively costly. On the other hand, with a $K_d$ value only fivefold less (10⁻³ cm s⁻¹) the leak rate would be just 40% higher than the rate of CO₂ fixation. At this $K_d$ value the predicted ATP requirement (5.4 ATP/CO₂ fixed) is approx. equivalent to that of C₄ photosynthesis (Edwards et al. 1976). With $K_d$ values lower than 10⁻³ cm s⁻¹ the predicted energy requirements become even more favorable. Zervirth and Kaplan (1981), using CO₂ efflux-rate analysis, estimated the $K_d$ for another unicellular green alga, *Dunaliella salina*, to be 10⁻⁴·10⁻⁵ cm s⁻¹. Even with a $K_d$ tenfold higher than their maximum estimate the energy requirements predicted by the model are relatively moderate. Although not conclusive, this analysis of the potential energy costs of the *Chlamydomonas* CO₂-concentrating system indicates that the system is no more energy costly than C₄ photosynthesis.

Support for this analysis of energy costs can be found in estimates of the relative quantum requirements of 5%-CO₂-adapted cells (no CO₂-concentrating pathway) and air-adapted cells (CO₂-concentrating pathway active). From the light-response curves for photosynthesis in these two cell types of *C. reinhardii* (Fig. 4), the relative quantum requirement of air-adapted cells was estimated to be approx. 20% higher than that of 5%-CO₂-adapted cells. These data would correspond to an ATP requirement of only approx. 4 ATP per CO₂ fixed. These experiments were necessarily performed with saturating CO₂ levels in order to obtain linear rates of photosynthesis at each light level, so not all CO₂ fixed by air-adapted cells was first transported. At air levels of CO₂, therefore, the quantum requirement for air-adapted cells would be expected to be even higher, but photorespiration would no doubt increase the quantum requirement for 5%-CO₂-adapted cells as well. Relative quantum requirements estimated...
in a similar manner for the cyanobacterium Synechococcus were found to be nearly twice as high for air-adapted cells relative to high CO₂-adapted cells (Badger and Andrews 1982). These data would correspond to a slightly higher but still modest increase in the ATP requirement (approx. 6 ATP/CO₂ fixed). These two estimates of relative quantum requirements support our conclusion that the CO₂-concentrating pathway of C. reinhardtii is probably similar to C₄ photosynthesis in its energy requirements.

There is some uncertainty as to whether HCO₃⁻ or CO₂ is the species actually taken up by Chlamydomonas (Berry et al. 1976; Tsuzuki 1983). However, considering the observations that both the ca-1 mutant and ethoxyzolamide-inhibited wild type are demonstrably CO₂-limited even with internal inorganic carbon concentrations as high as 10–15 mM, one cannot escape the conclusion that the accumulated inorganic carbon must be HCO₃⁻ rather than CO₂ (Spalding et al. 1985). This could be the case only if the species released into the cell (or the chloroplast) after transport was HCO₃⁻, since, under these conditions, CA would be unavailable to catalyze hydration of CO₂. With this point in mind it is unimportant to the model whether CO₂ or HCO₃⁻ is the substrate for the transporter. The Kᵣ for transport (Kᵣ) was actually determined as a Kᵣ for inorganic carbon under conditions where complete equilibration between CO₂ and HCO₃⁻ would be expected, rather than as a Kᵣ for HCO₃⁻. To deal with CO₂ as the substrate, Kᵣ would simply need to be reduced based on the ratio of CO₂ and HCO₃⁻ at pH 7.0. If CO₂ was not only the substrate for transport but was also the species released inside the cell, this would still have no appreciable effect on the model for wild type or the pmp-1 mutant because of CO₂-HCO₃⁻ equilibration inside and outside the cell. It would, however, seem impossible to explain the characteristics of the ca-1 mutant (or ethoxyzolamide-inhibited wild type) if CO₂ was the species transported and released inside the cell.

Our treatment of Chlamydomonas as a single compartment in this model is, of course, an oversimplification, since it is a eukaryotic alga. This simplification should not introduce much error, however, especially in the overall conclusions reached, if the assumption that transport occurs at the plasmalemma is made. Our overall conclusions should still hold even if transport was assumed to occur at the chloroplast envelope. The net effect of this last assumption would only be to put a membrane around our model cell. This would naturally introduce quantitative error into many of the predictions of the model, but our basic conclusions would remain valid.

Although the model of CO₂ assimilation presented in this paper uses relatively simple assumptions, it accurately predicts most of the photosynthetic characteristics of wild-type Chlamydomonas and three mutants defective in some portion of the CO₂-concentrating system. This ability to predict accurately the photosynthetic characteristics of the cells demonstrates that the CO₂-concentrating system, as depicted in the model, can explain the photosynthetic characteristics of this alga. It will now be possible to use this model and further refinements of it to make testable predictions about photosynthetic characteristics not discussed in this paper, such as the effect of pH on the photosynthetic CO₂-response curve or on the CO₂ compensation concentration.

Appendix

In considering the uncatalyzed dehydration of bicarbonate two reactions are important: the direct dehydration of the bicarbonate ion

\[ \text{HCO}_3^- \xrightarrow{k_a} \text{CO}_2 + \text{OH}^- \quad \text{eqn. 18} \]

and the direct dehydration of bicarbonate involving carbonic acid as an intermediate

\[ \text{H}^+ + \text{HCO}_3^- \rightarrow \text{H}_2\text{CO}_3, \quad \text{eqn. 19} \]

\[ \text{H}_2\text{CO}_3 \xrightarrow{k_c} \text{CO}_2 + \text{H}_2\text{O}. \quad \text{eqn. 20} \]

Since reaction 19 is almost instantaneous (Sirs 1958), the overall rate equation for the dehydration of bicarbonate to CO₂ is

\[ \frac{d[\text{CO}_2]}{dt} = k_a[\text{HCO}_3^-] + k_c[\text{H}_2\text{CO}_3] \quad \text{eqn. 21} \]

where \( k_a \) is the rate constant from reaction 18 and \( k_c \) is the rate constant from reaction 20. Since equilibration in reaction 19 is virtually instantaneous, \([\text{H}_2\text{CO}_3] \) can be considered as a constant function of \([\text{HCO}_3^-] \)

\[ [\text{H}_2\text{CO}_3] = \frac{[\text{HCO}_3^-][\text{H}^+]}{K_{eq}} \quad \text{eqn. 22} \]

where \( K_{eq} \) is the equilibrium constant for reaction 19. Combining equations 21 and 22 the overall rate equation for bicarbonate dehydration becomes

\[ \frac{d[\text{CO}_2]}{dt} = k_a[\text{HCO}_3^-] + \frac{k_c[\text{HCO}_3^-][\text{H}^+]}{K_{eq}}. \quad \text{eqn. 23} \]

Values taken from the literature for \( k_a \) and \( k_c \) are \( 2 \cdot 10^{-4} \text{ s}^{-1} \) and \( 18 \text{ s}^{-1} \) (Pocker and Bjorkquist
1977) and for $K_{eq}$ is $1.72 \cdot 10^{-4}$ (Wissburn et al. 1954). Inserting these values and solving for pH 7.5 we obtain a value of $3.5 \cdot 10^{-3}$ s$^{-1}$ for the overall rate constant $k_3$ for dehydration of bicarbonate to CO$_2$ at pH 7.5

$$\frac{d[CO_2]}{dt} = (3.51 \cdot 10^{-3} \text{ s}^{-1}) [HCO_3^-] \quad \text{eqn. 24}$$

Two reactions are also important in the uncatalyzed hydration of CO$_2$: the direct hydration of CO$_2$ by reaction with OH$^-$

$$CO_2 + OH^- \rightarrow k_i HCO_3^- \quad \text{eqn. 25}$$

and indirect hydration of CO$_2$ with carbonic acid as an intermediate

$$CO_2 + H_2O \rightarrow k_i HCO_3^- \quad \text{eqn. 26}$$

$$H_2CO_3 \rightarrow H^+ + HCO_3^- \quad \text{eqn. 27}$$

Since reaction 27 is almost instantaneous (Sirs 1958), the overall rate equation for hydration of CO$_2$ to bicarbonate is

$$\frac{d[HCO_3^-]}{dt} = k_b[CO_2][OH^-] + k_a[CO_2] \quad \text{eqn. 28}$$

where $k_b$, the rate constant from reaction 25, has a value of $8.5 \cdot 10^3$ M$^{-1}$ s$^{-1}$ and $k_a$, the rate constant from reaction 26, has a value of $3.7 \cdot 10^{-2}$ s$^{-1}$ (Wissburn et al. 1954). Inserting these values and solving for pH 7.5 we obtain a value of $3.97 \cdot 10^{-2}$ s$^{-1}$ for the overall rate constant $k_4$ for hydration of CO$_2$ to bicarbonate at pH 7.5

$$\frac{d[HCO_3^-]}{dt} = 3.97 \cdot 10^{-2} [CO_2] \quad \text{eqn. 29}$$

In order for the model to approach equilibrium with respect to internal CO$_2$ and bicarbonate concentrations the reactions involved in the interconversion of CO$_2$ and bicarbonate ($v_1$ and $v_2$, $v_3$ and $v_4$) must have the same equilibrium at the given pH (7.5). To achieve this for the uncatalyzed reactions, it was necessary to adjust either $k_3$ or $k_4$ so that the relationship

$$K_a = \frac{k_a}{[H^+] k_3} \quad \text{eqn. 30}$$

holds true, where $K_a$ is the dissociation constant for the first proton from the weak acid CO$_2$. The value used for $K_a$ was $4.266 \cdot 10^{-7}$ (pK$_a$ = 6.37, Umbreit et al. 1945), so at pH 7.5

$$k_a = 13.49, \quad \text{eqn. 31}$$

The value of $k_3$ was adjusted from $3.51 \cdot 10^{-3}$ s$^{-1}$ to $2.94 \cdot 10^{-3}$ s$^{-1}$, with the value of $k_4$ remaining $3.97 \cdot 10^{-2}$ s$^{-1}$.

For the carbonic-anhydrase-catalyzed hydration and dehydration reactions to have the same equilibrium as the uncatalyzed reactions the following relationship (from the Haldane equation)

$$\frac{V_{sat}K_{ba}}{V_{ba}K_{ca}} = \frac{k_a}{k_3} \quad \text{eqn. 32}$$

must hold true. This was accomplished by adjusting the value of $K_{ba}$ from 34000 to 30133 nmol ml$^{-1}$.

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