A Theory and Model for the Reaction of RuBP Carboxylase/Oxygenase and Photosynthesis in C3-Plants

Hadi Farazdaghi, http://www.farazdaghi.com, hadi@farazdaghi.com, GRC, CO_2 Assimilation in Plants:Genome to Biome, Aussois, France, Sept. 11-16, 2005

Abstract: Rubisco is a Storage/Sink protein, convertible to Enediol, that is the single enzyme for carboxylation. Rubisco-activase assembles the enzyme in light. A theory and model is advanced, introducing the conept that steady state photosynthesis is controlled by two steps. The first step is the synthesis of the enediol enzyme and the second step is carboxylation/oxygenation. It is found that the two-process theory of Farquhar et al is incompatible with the biochemical kinetics of the rubisco reaction. It is shown that photosynthesis is neither limited by Rubisco at low CO_2, nor by energy for RuBP regeneration at high CO_2.

Today's "widely used" Two-Process Model:
This model states that assimilation rate is limited by two independent processes: i) By the RuP2 saturated rate of **Rubisco at low p(CO_2)** and ii) By the rate allowed by **RuP2 regeneration** capacity at **high p(CO2)** (von Caemmerer & Farquhar 1981, Collatz et al 1990).

Limitations of the Two-Process Model:

1) Blackman Law: The velocity of a reaction will not change unless by increasing the concentration of the Limiting Factor.
2) Carboxylation responds to CO_2 at low CO_2, thus CO_2 is limiting at low CO_2 not Rubisco.
3) If Rubisco is limited at low CO_2, then photosynthesis stops at low CO_2. This is contrary to experimental observations.
4) The theory represents limitation of enediol at both high and low CO_2, while the Rubisco-limited component of the model uses Michaelis-Menten equation that is in conflict with the limitation of enzyme under low substrate levels. The theory results in no photosynthesis under both high and low CO_2.
5) While it is shown that Michaelis-Menten equation is only applicable to the transitional state, the model uses the equation for the steady state conditions. Both the mathematical and experimental validation of the model are invalid.
6) The model uses an empirical equation with an unidentified maximum, Jmax, supplemented by a variable curvature factor.
7) Yet the model produces systematic departure from actual data in cases with two or more response curves.

Concepts for the New Biochemical Model of Carboxylase/ Oxygenase.

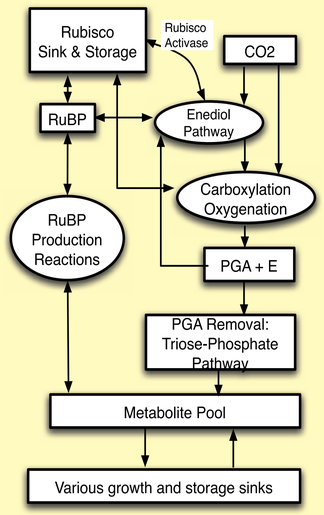
Carboxylation Rate is limited by **either CO_2 or the capacity of Enediol** Under All Conditions. **Enediol is the Single Enzyme Carboxylase-Oxygenase.** Before enediol there is NO Photosynthesis, and the TWO Processes of Farquhar et al reduce to a limitation of Enediol.

Photosynthesis is limited by either **Enediol** or **CO_2.** If Enediol is limited at $CO_2 = \Gamma$, then Photosynthesis Stops at $CO_2 = \Gamma$. The limitation of enediol is due to a limitation of either RuBP supply or Rubisco capacity.

The Function of Rubisco: Rubisco is a bi-functional protein, that is used as a **Sink** for the attraction and storage of RuBP that is to be released by Rubisco activase and combine later after carbamylation in enolization. The storage function maintains a supply of RuBP in the dark for the initial synthesis of enediol in light, before the start of Calvin cycle. This is why Rubisco is notoriously inefficient as a catalyst.

The Enediol Pathway: Enediol Pathway begins with the separation of the Rubisco protein from RuBP and other sugar-phosphates by Rubisco-Activase, and continues into carbamylation and binding of Mg++ metal ion until its combination with the co-factor RuBP and formation of enediol. The synthesis of enediol is initiated by Rubisco activase transitionally and is maintained interactively with triose-phosphate pathway that is responsible for the removal of PGA and and return of Rubisco for steady state carboxylation.

- An initial limitation of enediol can be due to a limitation of either Rubisco activase for the separation of Rubisco from RuBP, or a limitation of RuBP storage per Rubisco site, caused by the inhibition of other sugar phosphates such as CA1P.
- A limitation of Triose-Phosphate pathway can limit the steady state carboxylase activity by limiting the return of Rubisco through product inhibition.



The New Model: The activity of carboxylase is not independent of light or RuBP regeneration as considered by the two-process model (Farquhar et al 1980). Rubisco activation state is dependent on both Rubisco activase and Calvin cycle (Portis et al 1995).

1) The Transitional State: The velocity of carboxylase follows Michaelis-Menten equation with a maximum that is limited by the maximum capacity of Enediol, or the capacity of RuBP-saturated activated Rubisco (V_{cmax}).

$$1/V_c = 1/V_{cmax} + (1+O/K_o)/\Psi_c \; C \qquad (1)$$

where, $\Psi_c = V_{cmax} / K_c$

2) The Steady-State: The Enediol used in the transitional State must be retrieved in the form of its two constituent components: i) as Rubisco, from Triose-Phosphate Pathway(TPP) of Calvin cycle that removes PGA, and ii) as RuBP, from the RuBP-Regeneration Pathway (RRP) of the Calvin cycle.

Thus the biochemical reaction has two steps:

Step 1: Assembly and synthesis of Enediol enzyme form.

Step 2: Carboxylation of Enediol and production of enzyme intermediates up to production of PGA and release of Rubisco (see diagram above).

Under steady state conditions the protein Rubisco is divided into the two groups of enzyme components that participate in the two steps, but only the enzyme component that participates in the slower step will be limiting the reaction rate (Rate-Determining Step), and the rest of enzyme determines the maximum velocity of the reaction.

Therefore if $\alpha_1 V$ is the enzyme component that participates in Step 1, and $(1+\alpha_2)V$, the enzyme component that participates in Step 2, total enzyme will be $(1+\alpha_1+\alpha_2)V$, when both RuBP and CO_2 are saturating Rubisco. When CO_2 is limiting the second step we have:

$$(1+\alpha_2)\, V_c^2 - V_c\,[\,(1+\alpha_1+\alpha_2)\,\Psi_c\, C + V_{cmax}] + V_{cmax}\,\Psi_c\, C = 0 \qquad (2)$$

And when the synthesis of Enediol is limiting in the first step and the activation state of Rubisco is dependent on Rubisco activase, then:

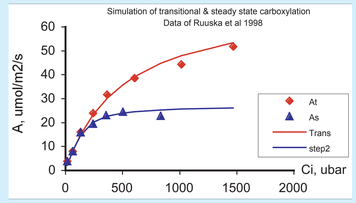
$$\alpha_1\, V_i^2 - V_i\,[(1+\alpha_1+\alpha_2)\,\phi\, I + V_{cmax}] + \phi\, I\, V_{cmax} = 0 \qquad (3)$$

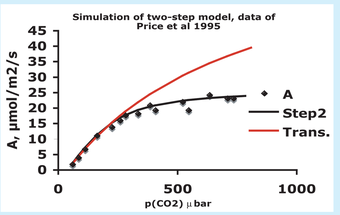
V_{max} represents the velocity allowed by total concentration of activated Rubisco and it maximum is V_{cmax}. When Enediol is rate-limiting due to a limitation in the triose-phosphate pathway for the return of Rubisco, the limitation will be shifted to the second step due to product inhibition by PGA, then:

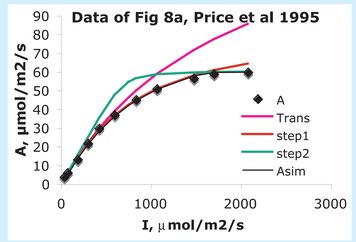
$$(1+\alpha_2)\, V_i^2 - V_i\,[(1+\alpha_1+\alpha_2)\,\phi\, I + V_{cmax}] + \phi\, I\, V_{cmax} = 0 \qquad (4)$$

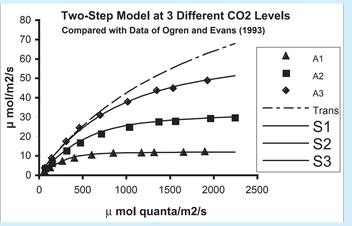
Model Validation

Simulation of photosynthesis is performed with the data of Ruuska et al (1998 panel 1, top left)), data of Price et al (1995 panels 2 & 3, top right & bottom left). Each panel uses the kinetic data of the respective authors. Panel 4(bottom right), data of Ogren & Evans (1993, courtesy of Dr. Erling Ogren).





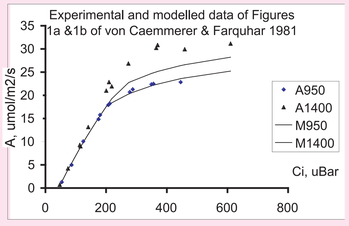




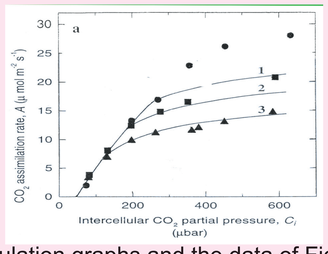
The model for each figure is a function of the main limiting factor for the steep part of the curve (eg. CO_2 for the top two figures and irradiance (I) for the bottom two figures) with a limitation at the top of the curve by enediol. We use the Michaelis-Menten equation, or rectangular hyperbola, to fit the initial velocities at low substrate levels.

The rest of the response curves follow either i) equations for the limitations of Step 1 followed by Step 2 for light response curves or ii) only the limitation equations of Step 2 for CO_2 response curves. Reductions in excess of the limitation of Step 2 is the result of Product Inhibition caused by additional limitations of triose-phosphate pathway. For the derivation of the two-step Rubisco reaction equations visit www.farazdaghi.com.

The model of Farquhar et al fits a single curve from a given experiment, via adjustment through a convexity factor and an empirical variable (J_{max}) derived from the experiment. For multiple CO_2 and radiation levels, the model provides systematic departure from experimental data. For example:



Data of Fig.1a of von Caemmerer & Farquhar (1981) are extracted and plotted against the simulation of Fig1b of the authors based on the instructions of the authors.



Simulation graphs and the data of Fig 2.18 and 2.19 respectively of von Caemmerer (2000) are electronically scanned, brought to the same scale and superimposed.

Conclusions: Understanding Rubisco as a bi-functional protein that stores RuBP and is convertible to the enediol carboxylase/oxygenase enzyme provides a simple mechansitic model of carboxylation that is in keeping with experimental observations. The new model is able to predict photosynthesis variations under different CO_2 and light levels, does not require empirical correction factors and requires fewer parameters than other models in use today.

The new concept of enzyme synthesis in light, and its dependence on both light and CO_2 through the Calvin cycle, provides a more accurate picture of the dynamics of photosynthesis.

The two steps of enzyme synthesis and carboxylation reaction can explain the differences observed under transitional and steady state conditions. The kinetic models presented for the separation of the two reaction steps and the new implementation of a Rate-Determining Step under limiting substrate conditions, and co-limitation under substrate saturation, have provided a mechanistic basis for further expansion of the model. The separation of the two steps also helps identify the factors that affect each step and their interactions with environmental parameters such as temperature, water deficit, and global carbon cycle and environmental change.