Comments

Annonymous wrote:

Hadi:
Is it not possible to use your substrate function for both light and CO2?
09/05/04 13:03:38

hadi wrote:

The simple answer is no. Based on the theory, the concept of rate determining step suggests that in a multi-step reaction only the slowest step determines the rate of reaction. Therefore only one enzyme-substrate complex can be limiting. This means that either a single substrate can be limitimg or the enzyme.
09/08/04 09:42:23

hadi wrote:

Based on my previous comment, a modification is necessary in the abstract in:
1) Consideration of two steps for the reaction, one for each "substrate".
The word "substrate" should be changed to "enzyme-substrate complex".
H.F.
09/16/04 16:40:27

hadi wrote:

I have received a number of interesting and thoughtful comments by email from some distinguished scholars with specific interests in activation and biochemical reaction of Rubisco. To the extent that it is beneficial to the readers, and protects the anonymity of the commentators, I would provide the comments and my answers gradually in the following paragraphs.
09/17/04 12:54:57

Comment Series 1: wrote:

A model based only on rubisco parameters is certainly a valid approach, but measurement of many of the required parameters is difficult - RuBP, PGA (for product inhibition that your model seems to use), inhibition constants, etc.
09/17/04 13:25:41

hadi wrote:

Answer: From equations 35 and 36 of the model, you may note that RuBP regeneration is considered to be either “energy-limited” or “Calvin cycle capacity-limited”. In either case RuBP is replaced with its equivalent energy. Therefore, measurements of RuBP concentration are not required.

Simulations of Fig 3a give examples of “energy-Limited” cases of RuBP regeneration, and all the parameters of model are calculated on the basis of measurements of initial conditions and maximum saturation velocities. No other intermediate value or any empirical constant is needed for the model.

Extra measurements of values near light saturation are needed for CO2 saturated carboxylation to establish possible limitations of Calvin cycle capacity on Vmax as shown in Fig. 2a.

It is important to note that, often our judgment of saturation may differ significantly from what the plants tell us. For example, the two-system theory of Farquhar et al assumes that, Rubisco is always saturated with RuBP, and ambient CO2 is sufficient to saturate Rubisco. Therefore, any deviation of A/Ci curve from Michaelis Menten curve in mid-day hours should be due to a limitation in RuBP regeneration of Calvin cycle. Thus, when Price et al (1995, Fig 6a), find a value of A = approx. 24 (see units) at Ci = 800, the limitation is considered due to inadequate RuBP regeneration from Calvin cycle, while CO2 and light are saturating. Yet, in the same paper, (Fig 8a) a much higher value for A of about 60, at external CO2 of 1750 is reported by the same authors and for the same plants. This rejects the saturation of CO2 for the first case (i.e for A=24). A similar Amax value of about 60, had also been previously reported by Masle et al (1993) for the same plants, that rejects an accidental one time error for the latter data.

09/19/04 23:06:39

comment series two: wrote:

I think the success of the Farquhar et al model is that the model parameters can be easily determined by various experimental methods for validation analysis. In your model you have sufficient parameters to "fit" or simulate any photosynthetic response curve but it is not obvious (to me at least) what sorts of measurements one would make to independently verify the values of the model parameters you obtain.

09/24/04 10:42:13

hadi wrote:

My best interpretation of this comment is that, I have not been clear enough in my presentation of the model and its comparison with that of Farquhar et al.

I still leave the full comparison to be made by other modelers and model users. But, briefly, there are several versions of the two-system model of Farquhar et al; such as, the integrated 1980 model, de Pury & Farquhar (1997) for the response to light, integrated model of von Caemmerer (2000), and a few others. There are at least two empirical parameters in these

models, Jmax and the convexity factor. The convexity factor is a coefficient that varies widely (Evans & Farquhar 1991, Ogren & Evans 1993, Ogren 1993) and according to Collatz et al (1990), it has no mechanistic meaning. There are no such parameters in my single-enzyme model. As mentioned under the “General Discussion”, if “V^2” and its coefficient are excluded from equation 47, the remaining part shows the kinetics of an equilibrium reaction; a1+a2 determine the magnitude of enzyme substrate complexes including the feedbacks relative to the last step, while “V^2” and its coefficients reflect the effect of Rate-Determining Steps and the irreversible link between the two steps. With all such details, yet there is no parameter in the model that requires additional effort in the measurements compared to Farquhar et al model. Indeed, the model reduces the number of parameters to be measured by establishing their internal relationship. For example, Ogren & Evans (1993) use three individual response curves, with more than three parameters for each, to describe a set of data for eucalyptus that could be described more elegantly and accurately, with fewer parameters as shown in Fig 3.
09/27/04 11:06:50

Comments series three wrote:

As best I can determine, neither the Farquhar et al. model or your model explicitly uses Rubisco activation state as a variable. This is one parameter that can be easily measured and ,……. Of course the downside is that the dependence of Rubisco activation state on many of the other parameters that one might like to have in a model are not always well known.
10/01/04 14:17:09

hadi wrote:

Answer: Well, as far as my model is concerned you are right. I do not use the term "Rubisco activation state" explicitly, because it may be interpreted very broadly, and out of the scope of this work. However, what I have said explicitly, and have used in my derivation of the model mathematically, (eqns 19 and 20), is that the Kcat of the reaction varies for different velocities. This is a quality distinctly different from that of Michaelis-Menten type models.
10/02/04 21:27:03

steve wrote:

Is your model based on product or feedback inhibition as mentioned in comment series 1?
10/28/04 11:26:52

hadi wrote:

A very good question. The answer is not a simple Yes or No. Let us look back at Fig. 2 first. Take the simulation for spinach; we have three curves, one shows the initial maximum reaction. This shows the maximum possible rate of reaction, when CO2 is not limiting; it varies with light, it is transitional and is limited by the Maximum Rubisco Capacity (Vcmax). This is consistent with the potential transitional carboxylation rate that Laisk (1985) and Ruuska et al (1988) have experimentally found and von Caemmerer (2000) is using in her calculations. The second curve gives the actual steady state rate of carboxylation when energy is limiting, and it seems to be equivalent to what is often considered as Rubisco activation state when CO2 is not limiting. This varies for different levels of carboxylation and its maximum is determined by the Rubisco activation state at light saturation (Vmax). Therefore, the limitation of the first step is certainly not due to feedback or product inhibition. For the lack of a better term, I would call it conformation inhibition, because I think it is mainly due to conformation of enzyme-RuBP complex and the ping-pong mechanism of Enol-RuBP. However, a side chain inhibition by xylulose-1,5 bisphosphate (McCurry, S.D., and Tolbert, N.E. 1977, J.Biol. Chem. 10:252:23:8344-6.) may have the same effect and can lend additional strength to this inhibition. The third curve shows the limitation of the second step. When CO2 is saturating, it reflects the other possible inhibitory factors in the second step, such as the effect of feedback or product inhibition by PGA. Therefore, when CO2 is limiting, such as in the cases given by Price et al (1995, Fig 6a & Fig. 9a), a consideration of a limitation in RuBP regeneration system cannot be supported logically and experimentally, as evidenced by Fig 8a of the same authors.
Therefore, at "Light Saturation" , when there is no response to CO2, the possibility of other limitations such as feedback inhibition by Calvin cycle metabolites can be explored.
10/29/04 17:15:00

Peter wrote:

I like your model. It seems to work well; however, it is not clear to me how you can explain the role of Rubisco activase here.
11/04/04 13:32:13

hadi wrote:

Thak you Peter. Rubisco activase is effective for enzyme activation-deactivation. This model starts on the assumption of full activation at first, and introduces the factors that cause de-activation through holding the enzyme in the forms which are not readily productive. Because of this assumption, the content of Rubisco activase and its contribution to the rate of reaction do not enter into the model directly.
11/08/04 15:27:00

Mark. wrote:

Hadi:

The model of Farquhar et al is known as Rubisco limited model. This name is used in the literature and is accepted by von Caemmerer 2000. Is there any good reason for the change of name to Two-Limitation model?

Mark.

11/12/04 08:01:13

hadi wrote:

Hi Mark;

Thank you for your question. The fact is that, except for the paper of Carl Bernacchi and his colleagues (Plant Cell & Environ 2001, 24:253-9), I have not come across such references. What I mave most commonly observed has been "Rubisco-limited photosynthesis" for the steep part of the CO2 response curve as opposed to "RuBP limited photosynthesis" for the plateau of the curve. I did not find a reference to "Rubisco-Limited model" in von Caemmerer 2000. If such name, or any other name is preferred by the model developers I would certainly use it. The term "two-limitation" model was used for the lack of a better name, and was proposed by one of the prominent contributors to the Farquhar et al school of thought.

11/12/04 23:48:55

Peter wrote:

Hello again Hadi:

Correct me if I am wrong please. The difference between your Step 1 and Step 2 models is that, if a1=0 then model 1 will be changed to Michaelis Menten equation, while if a2=0, the model for step 2 will not change. Is this a correct interpretation?

11/25/04 02:02:00

hadi wrote:

Hello Peter. This is a very good question. If a1=0 then you are mathematically correct. However, biochemically, when step 1 is limiting, a1 =k9/k3 should be larger than 1+a2, therefore, it cannot be equal to zero. Because of this, when a1 and a2 are at their minimum values (a1=1, a2=0), the minimum value for “a” in equation 40 is 0.5 as shown in equation 43. In single-substrate Michaelis Menten model, the physical phase of random collision between the substrate and enzyme is assumed to be much slower than the biochemical phase of product formation and release. Because of this, it is possible to assume that the amount of enzyme engaged in the biochemical phase is negligible relative to the total concentration of enzyme. However, in two-substrate ordered reactions, there are two such combined steps. Although, due to Rate-Determining

Step, we consider a single substrate equation for the limitation of the slower step, we cannot totally ignore the enzyme that is engaged with the faster step, particularly when enzyme becomes a limiting factor. Therefor, in general, two-substrate ordered reactions, such as carboxylation or oxygenation do not follow Michaelis-Menten type models for steady state conditions.
11/25/04 10:35:24

Stan wrote:

Hadi:
Can you compare your model with Rubisco model of Mott & Woodrow?
12/11/04 12:16:13

hadi wrote:

Hi Stan: Two different directions; Mott & Woodrow start with Rubisco activation and go through the transitional reaction rate to come to carboxylation. I start with activated enzyme and model the carboxylation. I intend to add a component also for enzyme activation, although it does not materially change the model. Perhaps a comparison can be more meaningful after that.
thanks anyway.
12/11/04 12:47:08

Rob wrote:

Hi
I am a grad student and a bit mixed in who says what. is this correct?
Farquhar et al: steep part of the response to CO2 is rubisco limited and the phateau RuBP regeneration limited.
Farazdaghi:steep part of the same curve CO2 limited, and the plateau enzyme?
12/11/04 13:28:47

hadi wrote:

Hi Rob:
You are correct in both cases.
In the case of my work, two response curves are needed for complete analysis: One curve for response to CO2 at RuBP (represented by radiation) saturation, and another curve for the response to RuBP at CO2 saturation. With such curves the plateau is always limited by Rubisco enzyme, either directly through co-limitation of enzyme-substrate complexes of the two substrates, or indirectly through side chains (eg xylulose bisphosphate, ...) or feedback inhibition by PGA.
12/16/04 08:17:10

Nick wrote:

Sir:

According to your theory the initial rate of carboxylation follows Michaelis-Menten equation. Does it not mean that Michaelis-Menten controls the activated level of Rubisco too?

Nick.

12/29/04 12:10:13

hadi wrote:

Thank you for the question Nick.

Yes it does, but for the tramsitional rate only when the total enzyme participates in production of the maximum rate of reaction (Vcmax). But, under steady state conditions, the enzyme-substrate complex of the slower step limits the rate of release of free enzyme upon which Vcmax is dependent.

01/02/05 01:49:33

Jerry wrote:

It is interesting that the approximate versions of your model for light and CO2 turn into Blackman type equations with or without the convexity factor respectively. Can you elaborate on the differences of your model under these conditions with the Blackman or Farquhar et al model please?

Thanks;

Jerry

01/21/05 10:34:27

hadi wrote:

Thank you for your question Jerry. Indeed, equations 43 and 44 are the Blackman look alike equations; the coefficients are approximations that are difficult to trace back to their origins without knowledge of their history and the assumptions made for their derivations. The use of such models cannot be encouraged, except for use as a preliminary guideline when one knows the limits of the application. As an example about the limits of validity, I have given equation 46 as a close approximation of net photosynthesis from rectangular hyperbola for the transitional step. For this purpose, I think the equation can provide reliable outputs. But for equations 43 and 44 the degree of approximation particularly with respect to the limits and extent of interactions is not clear at this stage. This is because the coefficients that are omitted or approximated in equations 40, 41 and 42 are related to the distribution of enzyme between the limiting “enzyme form” (rate-determining step) and the rest of the enzyme for which we do not have complete information. Further work on these coefficients can help scientists to a better understanding of the origins of the inefficiencies of Rubisco, and perhaps to a better design for enzyme.

The next part, about a comparison with the model of Farquhar et al, this has also come up in some private communications, Originally, I thought that I had given my views in brief and my preference was that other interested researchers and scholars pursue the subject matter. However, I may have to expand the subject matter further myself.
01/27/05 13:25:36

Jerry wrote:

Hi, Thank you for your response. However, you mentioned that the "rate-determining step" (RDS) is dependent on the limiting "enzyme form". In other references, RDS is said to be related to either Calvin cycle or Rubisco. Would you comment on the differences please? Thanks.
02/11/05 12:04:45

hadi wrote:

Jerry:
Thank you for your question and comments.
I have used the term “rate-determining step” only for the limiting component of the velocity of reaction within the boundaries of one enzyme. I preferred the use of the term “enzyme form” over “substrate” limitation, in order to keep the reader conscious that the enzyme is also a component, that is associated with the limiting factor and may cause feedback and limitation for other components.
In a chain of multi-substrate, multi-enzyme (a multi-currency system) such as Calvin cycle, the analysis of system can be made for the segments (subsystems) that can be secured for identification of their inputs and outputs. Calvin cycle, representing the dark reaction, can be divided into a few subsystems for analysis, which include Rubisco, triose-phosphate pathway, and RuBP regeneration pathway. However, sometimes the subject is a comparison of Rubisco and Calvin cycle, in that case Calvin cycle could include both triose-phosphate and RuBP regeneration pathways. Therefore, it is difficult to decide who or what model is right without going into the specifics of the subject matter.
02/23/05 16:15:24

Nico wrote:

Have you published your article in any journal? In case I would want to include it in an article on coffee photosynthesis I’m currently writing .
02/24/05 11:15:57

hadi wrote:

Nico:
Thank you for your interest. Internet publications are treated in the same way as conference papers. therefore you can refer to the paper as:

Farazdaghi, Hadi (2004) A theory ....
http://www.farazdaghi.com
good luck with your article
02/26/05 11:22:57

hadi wrote:

Because of the increase in the length of the questions and comments section, longer discussions are removed, and will be included in the articles that are planned for future dates, one on Rubisco activation state and the other on the limitations of the biochemical theory and the two components of the model of Farquhar, von Caemmerer and Berry (1980), von Caemmerer and Farquhar (1981). Other questions and comments will be welcomed.
06/19/05 02:17:46

hadi wrote:

I have received a considerable number of emails to return the comments and remove them only after my review of Farquhar et al model is posted. So, I will do accordingly.

The question of steve I have described, but the question of Hugh was my critique of the model of Farquhar et al. His comment was that if it were that simple, how come no body noticed that in the past 25 years?
06/23/05 23:39:21

hadi wrote:

A reader had asked about the Rubisco-limited models and the difference, if any, between the definition of Rubisco limitation between the two models of Mott & Woodrow, and Farquhar et al. Unfortunately, by some error, the actual question of the reader is deleted. With apologies to this reader that the exact wording of the question is not kept, the answer is given below:

Hi Steve-

Thank you for your comment and question.

Indeed, you are correct, there is a world of difference between the two Rubisco-limited definitions in modeling photosynthesis. The differences are based on what the models represent. Mott & Woodrow's Rubisco limitation represents the activation of Rubisco by Rubisco activase and radiation, which influences both Rubisco activity and RuBP regeneration through Calvin cycle.

This is meaningful theoretically and defendable experimentally, but most importantly, contrary to the Rubisco-limited assumptions of Farquhar et al, their conclusion is enzyme-specific and limited only to Rubisco. They do not make generalizations that may extend to other enzymes.

Farquhar et al (1980) consider a fully activated RuBP saturated Rubisco, and assume that its reaction rate, is Rubisco-limited at low P(CO2). Well,

this is their assumption for fully activated Rubisco and it is possible to debate the validity of this assumption.

But the problem gains a universal dimension when von Caemmerer & Farquhar (1981) calculate the derivative of the Michaelis-Menten equation. They use high school mathematics to show that initial slope is correlated to Vcmax.

The authors present this relationship as a mathematical proof for validation of their theory that carboxylation rate is limited by Rubisco because the initial slope is Rubisco-limited.

This is contrary to the fact that the rate of carboxylation is correlated with CO2, and therefore, according to both Blackman and Michaelis-Menten models, carboxylation rate must be CO2 limited.

The mathematical interpretation of von Caemmerer and Farquhar defies the basic principles of mathematics. This apparently seems to be immaterial to these modelers, as Collatz, Berry, Farquhar and Pierce (1990) in response to the previous version of this model by Farazdaghi & Edwards (1988) state:

"This model (Farquhar et al) is based on a framework of assumptions rather a rigid mathematical formulation like the" Farazdaghi & Edwards model."

The mathematical interpretation of von Caemmerer & Farquhar (1981) is uniquely incorrect. If it were correct, all the reactions with fully activated enzymes that follow Michaelis-Menten kinetics must have been declared enzyme-limited at their lowest substrate concentration. So, it means that all fully activated Michaelis-Menten reactions will have to be most severely substrate-limited.

I intend to provide a broader review of this model in future months.

06/23/05 23:42:42

hadi wrote:

Hi Hugh:

Thank you for your question.

The theory of Farquhar et al looks very simple and "at first sight" is very convincing. It is not possible to talk critically about a model that is widely used without hurting some model users. However, any incomplete discussion would not be helpful to the model users either. In the past, criticisms have been brushed off with some fine, seemingly scientific, maneuver. If the past can be a lesson for the future, what is that lesson?

The problem of the model users is not that simple. Many do not have the mathematical background to examine the fundamentals of a model. The criteria for judging a model for some of these model users is faith on the authority of the modelers, particularly if this criteria is combined with two magic words: "it fits" or "it works". This is exactly what the architects of

this widely used model have been preaching. Collatz, Berry, Farquhar & Pierce (1990) in review of the previous version of our model (Farazdaghi & Edward 1988) and promotion of the model of Farquhar et al, emphasize that their model works, though with the help of an empirical convexity factor. They recommend that: Do not fix something that works. There is apparently no attention to “how it works” or “why it works”.

But, in fairness to the model users, it may be difficult to note the delicate value-exchanges in the abstract of von Caemmerer and Farquhar (1981) in which from statement #1, statement #2 is concluded:

1.-"...the initial slope of the response of CO2 assimilation rate to intercellular p(CO2) could be correlated to in vitro measurements of RuP2 carboxylase activity….”

2.-"…These results are consistent with the hypothesis that CO2 assimilation rate is limited by the RuP2 saturated rate of the RuP2 carboxylase oxygenase at low intercellular p(CO2)...".

Simple language translation: 1- the initial slope is proportional to Rubisco activity (this is an incorrect statement, based on equation A16, initial slope is proportional to Vcmax).

In statement 2, they conclude that, CO2 assimilation rate at low CO2 is limited by Rubisco. This statement is incorrect, based on either the original or corrected versions of statement 1. This is just a confusing mathematical and biochemical statement in either case.

In the hierarchy of limitations of Michaelis-Menten equation, its maximum velocity constitutes the second level of limitations, while its independent variable, that is the substrate (CO2), constitutes the first level of the limitations.

It is clear that “Rubisco-limited hypothesis” is incorrect, both mathematically and biochemically, otherwise the principles of biochemistry and enzyme kinetics would have been changed by now. What is not clear and difficult to understand or ignore, is that the model of Farquhar et al rejects the principles of Michaelis-Menten in its Rubisco-limited theory, but uses Michaelis-Menten equation in that same model. It is not possible to have it both ways.

06/23/05 23:46:38

Martin wrote:

Hello Hadi,

In your answer to the first question you consider that in a multi-substrate reaction only one enzyme-substrate complex can be limiting and that woud be the rate-determining step. Now, let E be the enzyme-substrate form of the first step in a two substrate reaction. In a reaction with the second substrate (B), K1.E.B=(k2+k3)EB; the rate of reaction V=k3.EB is limited by EB or by either E or B. If the limitation of E is similar to the

limitation of Rubisco in Rubisco-limited hypothesis, what is wrong with it.
07/04/05 15:28:55

hadi wrote:

Hello Martin:

Thank you for your question. If my interpretation is correct, your E can be similar to fully activated RuBP saturated Rubisco, and you want to see what is wrong with limitations of either RuBP saturated Rubisco or CO2.

My answer is that there is nothing wrong with this, and that is why it has been so confusing. In fact this is the perception of most users of this model. Interestingly, the hypothesis of Farquhar et al is slightly different from this. Let's look at von Caemmerer & Farquhar (1981). At the end of the abstract you see:

"…. These results are consistent with the hypothesis that CO2 assimilation rate is limited by the RuP2 saturated rate of the RuP2 carboxylase-oxygenase at low intercellular p(CO2) and by the rate allowed by RuP2 regeneration capacity at high intercellular p(CO2)."

Now, if you check closely, you find that the hypothesis considers Rubisco limitation "at low p(CO2) and…" This is slightly different from your view of limitation of "either RuBisco or CO2", but it makes a drastic difference in the outcome of hypothesis. A limitation of Rubisco at low CO2 does not allow carboxylation to increase any further, no matter how much you increase p(CO2). In fact Rubisco would still be the limiting factor "at high intercellular p(CO2), which invalidates the second part of the hypothesis.

Indeed, the phrase of "Rubisco limitation at low CO2" is contradictory from within. At low CO2, Co2 is limiting, and when Rubisco is limiting, the reaction reaches its saturation level. The condition imposed by this phrase is impossible to meet.

If fully activated RuBP saturated Rubisco is limited at CO2 compensation point, it means zero net photosynthesis at any CO2 concentration, zero plant growth, zero animal growth. This theory cannot be valid.
07/05/05 18:52:41

Cal wrote:

Hi Hadi:

Thank you for your very inspiring comments. In response to Hugh, I would like to add, if I may, that the model users have always been seeking help. Look at the following S.O.S. message. It is public, from the internet.

"The MEDRUSH Vegetation Model C.P. Osborne and F.I. Woodward Dept. of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, U.K. August 1999

Please notify the authors of any errors in this description - contact

c.p.osborne@sheffield.ac.uk
The widely used model of Farquhar et al. (1980) describes leaf CO2-exchange, where steady-state photosynthesis is controlled by either the capacity to regenerate Ribulose-1,5bisphosphate (RubP) or the carboxylation capacity of RubP carboxylase / oxygenase"
Thanks again.
Cal
07/07/05 07:18:45

hadi wrote:
Hi Cal,
Rubisco reacts with both RuBP and CO2; with RuBP binding first and CO2 next. Thus, if it is a limitation of either Rubisco or RuBP regeneration, it comes back to the limitation of RuBP saturated Rubisco at low CO2 levels and RuBP at high CO2 levels. It does not make any difference.
What I can say is that Rubisco can never be limiting at low CO2, i.e. Both parts of the theory of Farquhar et al are invalid.
07/07/05 11:22:11

Brad wrote:
Hello Hadi:
I have seen another interpretation of the model of Farquhar et al, that is:
The steep part of the response of assimilation rate to p(CO2) follows the RuBP saturated kinetics of Rubisco, but the flat part is RuBP limited.
What is your opinion?
Thanks
07/17/05 12:33:44