The single-process biochemical reaction of Rubisco: A unified theory and model with the effects of irradiance, CO_2 and rate-limiting step on the kinetics of C_3 and C_4 photosynthesis from gas exchange

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ABSTRACT

Photosynthesis is the origin of oxygenic life on the planet, and its models are the core of all models of plant biology, agriculture, environmental quality and global climate change. A theory is presented here, based on *single process* biochemical reactions of Rubisco, recognizing that: In the light, Rubisco activase helps separate Rubisco from the stored ribulose-1,5-bisphosphate (RuBP), activates Rubisco with carbamylation and addition of Mg^{2+}, and then produces two products, in two steps: (Step 1) Reaction of Rubisco with RuBP produces a Rubisco-enediol complex, which is the carboxylase–oxygenase enzyme (Enco) and (Step 2) Enco captures CO_2 and/or O_2 and produces intermediate products leading to production and release of 3-phosphoglycerate (PGA) and Rubisco. PGA interactively controls (1) the carboxylation–oxygenation, (2) electron transport, and (3) triosephosphate pathway of the Calvin–Benson cycle that leads to the release of glucose and regeneration of RuBP. Initially, the total enzyme participates in the two steps of the reaction transitionally and its rate follows Michaelis–Menten kinetics. But, for a continuous steady state, Rubisco must be divided into two concurrently active segments for the two steps. This causes a deviation of the steady state from the transitional rate. Kinetic models are developed that integrate the transitional and the steady state reactions. They are tested and successfully validated with verifiable experimental data. The single-process theory is compared to the widely used two-process theory of Farquhar et al. (1980. Planta 149, 78–90), which assumes that the carboxylation rate is either Rubisco-limited at low CO_2 levels such as CO_2 compensation point, or RuBP regeneration-limited at high CO_2. Since the photosynthesis rate cannot increase beyond the two-process theory's Rubisco limit at the CO_2 compensation point, net photosynthesis cannot increase above zero in daylight, and since there is always respiration at night, it leads to progressively negative daily CO_2 fixation with no possibility of oxygenic life on the planet. The Rubisco-limited theory at low CO_2 also contradicts all experimental evidence for low substrate reactions, and for all known enzymes, Rubisco included.

1. Introduction

Carbon flux into the biosphere is mainly controlled by the global activity of Rubisco (ribulose 1,5-bisphosphate carboxylase oxygenase EC 4.1.1.39) (Falkowski et al., 2000; Cox et al., 2000). Rubisco is the enzyme that facilitates the entry of CO_2 into metabolism of plants and microorganisms through photosynthesis. The assimilation of CO_2 produces molecular oxygen (O_2), which is released to the atmosphere and maintains its CO_2/O_2 balance (Igamberdiev and Lea, 2006). The large majority of global CO_2 assimilation occurs in C_3 plants in which Rubisco operates at relatively low carboxylation efficiency, in addition to oxygen inhibition and deficiency of CO_2 as the substrate (Spreitzer and Salvucci, 2002).

Atmospheric O_2 can also react with Rubisco in place of CO_2 as a competitor for the same enzyme site (Ogren and Bowes, 1971). The supply of CO_2 to the Rubisco site is either direct in gaseous form

(in C_3 plants), or the atmospheric CO_2 is first transformed into C_4 acids in one compartment of the plant tissue, and then transported and released by decarboxylation in another compartment where Rubisco is located. The enzyme responsible for this biological pump, which produces a higher CO_2 partial pressure, is phosphoenolpyruvate carboxylase (PEPC) (Furbank and Hatch, 1987). There are three biochemical subtypes of C_4 plants, which differ in their C_4 acid decarboxylases used: NADP-malic enzyme (NADP-ME), NAD-malic enzyme (NAD-ME), and phosphoenolpyruvate carboxykinase (PEPCK) (Kanai and Edwards, 1999). By elevating the concentration of CO_2 at the site of Rubisco, the biochemical CO_2 pump of C_4 pathway increases the supply of CO_2 to Rubisco and suppresses oxygenation by increasing the competitiveness of CO_2 (Furbank and Hatch, 1987). In fact as Furbank and Taylor (1995) point out, "The C_4 pathway is a complex adaptation of the C_3 pathway that overcomes the limitation of photorespiration."

Photosynthesis is the essential core module of most large biosystem models, and Rubisco is the gateway to photosynthesis and oxygenic life under all conditions. Since a biochemical model of Rubisco reaction can be shared in photosynthesis of C_3 and C_4 plants, it can play a significant role in the development of biosystem models. Because of the complexity and interactions of inputs and outputs in Rubisco reaction, larger models that include prior and subsequent steps of photosynthesis cannot bypass this most significant step or suffice by a minimal treatment of the subject. Therefore, there is a need for a model that can reflect the complexities of the basic biochemical process of Rubisco and its relationship with electron transport system (ETS) and Calvin cycle, which at the same time allows necessary extensions to cover inhibitions and the effects of other variables such as environmental and genetic factors.

In this paper the function of Rubisco, and its relationships with its substrates, products, RuBP regeneration and electron transport systems, to the extent that they affect the core of the biochemical modeling of photosynthesis, will be analyzed and briefly discussed. New models will be presented that include two steps: Step 1, for the synthesis of Rubisco-enediol that is the real carboxylase/oxygenase, and Step 2, for the carboxylation/oxygenation of enediol. The roles of the electron transport system that ignites the process through activation of Rubisco activase and also provides fuel for the engine of Calvin cycle for continuation of the process will be addressed in the models. The effects of rate limiting steps will be considered in the models. The models will then be tested with verifiable published experimental data from the literature. The limitations of the two-process theory and model of Farquhar et al. (1980) and its subsequent modifications and clarifications by the senior architects of the model (von Caemmerer and Farquhar, 1981; Collatz et al., 1990; Price et al., 1995; Ruuska et al., 1998; von Caemmerer, 2000; Sharkey et al., 2007; von Caemmerer et al., 2009) will be discussed. The theory and models that are presented here are the refined results after a long period of exposure and comments. The goal of these early reviews was to receive feedback and avoid the shortcomings of others (Farquhar et al., 1980; von Caemmerer and Farquhar, 1981; Sharkey et al., 2007; von Caemmerer et al., 2009), which will be discussed later in this paper during the analysis of the two-process model.

2. A Historical Perspective

Biochemical models of photosynthesis have been favoured as the most robust scientific method for describing the quantitative relationship between biomass production and the environmental factors. Earlier models were based on Blackman (1905) law of single factor limitation. But with the advancement of biochemical kinetic principles of Michaelis and Menten (1913) and its extension to the two-substrate ordered reactions by Haldane (1930), these equations and rectangular hyperbola were used for a single substrate reaction of CO_2 fixation and the response of photosynthesis to irradiance respectively (Rabinowich, 1951), while versions of Haldane (1930) were used for the two-substrate ordered reaction of Rubisco with respect to RuBP. However, Farazdaghi and Edwards (1988) considered that since RuBP regeneration is dependent on PGA and energy from ETS, and the PGA that is produced by carboxylation is always greater than the amount required for the replacement of consumed RuBP, therefore ETS is the main limiting factor for RuBP regeneration. But, Farazdaghi (2007, 2009) revised this position. He argued that the bulk of energy is used by TP pathway, and any limitation of energy for this pathway will result in a reduction in the processing of PGA, as well as RuBP pool size, which has a feedback to, and inhibits Rubisco (Price et al., 1995). Therefore RuBP regeneration never directly affects the *steady state rate* of Rubisco reaction, so Radiation should be used as a factor that helps release E from PGA and maintain the activation state of Rubisco, as well as the RuBP supply.

It was shown by Farazdaghi (2009) that the Michaelis–Menten equation is also a special case of the Liebig law of minimum and Blackman (1905) law of limiting factors for enzyme reactions. In the Michaelis–Menten model, the rate of reaction is proportional to the concentration of enzyme–substrate complex, which is determined by both the concentrations of substrate and free enzyme. However, as the concentration of substrate increases, the concentration of enzyme–substrate complex also increases, which results in a decrease in the amount of free enzyme, thus the substrate and enzyme become co-limiting. Co-limitation continues until the amount of free enzyme becomes the limiting factor (at substrate saturation) and the rate of reaction does not increase any further (maximum velocity). Therefore, what determines the *limitation of enzyme for the reaction rate* is attainment of the maximum, or limit to the velocity, which is in agreement with both Blackman (1905) and Sharkey (1989). *It is important to note that the discussion of limitation is related to the limitation of the rate of reaction by the factor that is in short supply, and the limitation will be removed, if the supply of the factor is increased beyond its limiting concentration.*

Michaelis–Menten type models and their equivalent rectangular hyperbolae were used for the relationship between photosynthesis and CO_2 concentration, or radiation, with some degree of success (Hesketh and Moss, 1963). van Bavel (1975) combined the effects of both radiation and CO_2 as two Michaelis functions. A similar equation with respect to RuBP and CO_2 was also given by Badger and Collatz (1977). However, experimental evidence showed that in some cases the experimental curve developed a plateau faster than either rectangular hyperbola or Michaelis–Menten curves (Lilley and Walker, 1975; Chartier and Priol, 1976; Prioul and Chartier, 1977), and steady state CO_2 assimilation rate saturated "more quickly than can be predicted from the RuBP saturated CO_2 assimilation rate alone" (von Caemmerer et al., 2009). Thus, attention was directed toward explanations and alternative theories for describing CO_2 fixation in plants. One group of researchers used some empirical Blackman type models with different reasons for justification (Thornley, 1976; Prioul and Chartier, 1977). Other researchers noted that in two-substrate reactions, there were two types of reactions: (1) pre-steady state or transitional reaction that followed Michaelis–Menten kinetics and (2) steady state reaction that had a lower plateau (Jamin et al., 1991; Moulis et al., 1991). Laisk (1985), Laisk and Oja (1998) and Ruuska et al. (1998), confirmed these results for Rubisco. Laisk (1985) and Ruuska et al. (1998) showed that the steady state maximum velocity V_{max} was about 30% of the transitional maximum velocity (V_{Cmax}). The reason for this inhibition was unknown.

However, Laisk (1977) and Farquhar et al. (1980) independently presented a model with the standard biochemical formulation, but with a new assumption that considered "two independent