Plant Science 226 (2014) 61–70

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Rubisco catalytic properties optimized for present and future climatic conditions

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ARTICLE INFO

Article history:
Received 10 October 2013
Received in revised form 6 December 2013
Accepted 20 January 2014
Available online 31 January 2014

Keywords:
Climate change
Crops productivity
Photosynthesis
Rubisco
Water stress

ABSTRACT

Because of its catalytic inefficiencies, Rubisco is the most obvious target for improvement to enhance the photosynthetic capacity of plants. Two hypotheses are tested in the present work: (1) existing Rubiscos have optimal kinetic properties to maximize photosynthetic carbon assimilation in existing higher plants; (2) current knowledge allows proposal of changes to kinetic properties to make Rubiscos more suited to changed conditions in chloroplasts that are likely to occur with climate change. The catalytic mechanism of Rubisco results in higher catalytic rates of carboxylation being associated with decreased affinity for CO_2, so that selection for different environments involves a trade-off between these two properties. The simulations performed in this study confirm that the optimality of Rubisco kinetics depends on the species and the environmental conditions. In particular, environmental drivers affecting the CO_2 availability for carboxylation (C_c) or directly shifting the photosynthetic limitations between Rubisco and RuBP regeneration determine to what extend Rubisco kinetics are optimally suited to maximize CO_2 assimilation rate. In general, modeled values for optimal kinetic reflect the predominant environmental conditions currently encountered by the species in the field. Under future climatic conditions, photosynthetic CO_2 assimilation will be limited by RuBP-regeneration, especially in the absence of water stress, the largest rise in [CO_2] and the lowest increases in temperature. Under these conditions, the model predicts that optimal Rubisco should have high $S_{c/o}$ and low k_{cat}^{c}.

1. Introduction

The enzyme Rubisco catalyses the assimilation of CO_2 by the carboxylation of RuBP in the Calvin Cycle, and is therefore the most obvious target to improve the photosynthetic capacity of crops. Rubisco displays catalytic inefficiencies, including slow catalysis and imperfect discrimination between CO_2 and O_2 [1]. These inefficiencies not only limit the rate of CO_2 fixation, but also compromise the capacity of crops to use resources optimally, particularly water and nitrogen [1,2]. In principle, overcoming these limitations could be successfully accomplished by molecular interventions in the genes coding for both the large (LSu) and the small (SSu) subunits of Rubisco [1,3–5]. Among these interventions, replacement of crop Rubiscos by other versions of the enzyme with better catalytic performance has recently been tested and provides a powerful and promising approach [6–8]. However, the success – in terms of photosynthetic improvement – of the Rubisco replacement strategy will depend on the discovery of more efficient and compatible versions of the enzyme. As an example, Ref. [9] illustrated how Rubiscos from red algae would perform better than tobacco native Rubisco if they could be transferred into the chloroplast of tobacco.

A number of surveys have reported substantial variability among species in the kinetic parameters of Rubisco, the relative specificity for CO_2/O_2 ($S_{c/o}$), the Michaelis–Menten constants for CO_2 (K_c) and O_2 (K_o), and the maximum turnover of carboxylation (k_{cat}^{c}) [5,10–15]. Among the potential forces driving evolution of Rubisco catalysis, the availability of CO_2 for ribulose-1,5-biphosphate (RuBP) carboxylation has been proposed as a major factor, explaining some of the differences among distant phylogenetic groups, between C_3 and C_4 species and among C_3

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0168-9452/$ – see front matter © 2014 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.plantsci.2014.01.008

plants [12,16,17]. Thus, those environments where plants have evolved under high temperatures and low soil water availability should be prioritized in the search for better versions of the enzyme [18]. Under these conditions, the CO_2/O_2 concentration at the site of carboxylation is decreased due to lower leaf conductances in response to water scarcity and lower CO_2/O_2 solubility ratios as temperature increases [19]. It has been demonstrated that Rubisco has evolved toward higher $S_{c/o}$ under these conditions, reducing RuBP oxygenation and favoring the carboxylase reaction [13]. Nevertheless, comparisons of the scarce data on Rubisco kinetics – particularly data describing the full set of kinetic parameters on the same species – indicate a strong, negative correlation between $S_{c/o}$ and k^c_{cat}. Therefore, the suggestion that Rubiscos with high $S_{c/o}$ from extreme environments would allow higher CO_2 assimilation rates when transferred into the chloroplast of a crop plant grown under non-stressed conditions, and perhaps even in dense canopies where light limits photosynthesis, is doubtful. The maximum leaf conductances typically measured under optimal growth conditions provide maximum CO_2 availability at the site of carboxylation [2]. Under these conditions, a Rubisco with a higher k^c_{cat}, although at the expenses of a lower $S_{c/o}$, may provide the higher benefits [20,21].

The significant correlations between k^c_{cat} and $S_{c/o}$ – and K_o – have led to the opinion that Rubisco is nearly perfectly adapted for differing CO_2/O_2 concentrations and thermal conditions in the chloroplast environment [14,20,22]. Structural and mechanistic constraints have not allowed the development – either naturally or artificially – of *the perfect* Rubisco with increased specificity to CO_2 and a high rate of carboxylation. Natural evolution has resulted instead in Rubiscos in which there is a compromise between CO_2/O_2 affinities and the maximum rate of catalytic turnover dependent on the habitat and climate. This tradeoff is evident from a close inspection of published data which shows a significant scatter in Rubisco kinetics values and consequently in carboxylase catalytic efficiency (k^c_{cat}/K_c) [5,14,22]. Much less is known about the oxygenase kinetic parameters, although some correlation between oxygenase and carboxylase catalytic constants has been observed [23].

Maximum agricultural yields are obtained by growing crops with non-limiting resources by extensive irrigation and fertilization practices. However, the environmental footprint of intensive agriculture and the predictions for higher global temperatures and lower water availability for most current cropping areas demand novel solutions toward a more sustainable agriculture. It is widely believed that a more efficient Rubisco would provide not only more photoassimilates for the plant growth, but importantly do this at a lower cost of water and nutrients [24,25]. However, given the tradeoff between k^c_{cat} and $S_{c/o}$, it is not immediately evident what *more efficient* means in environmental and physiological contexts. Within this context, the present paper aims at the following: (i) to model how temperature and CO_2 and O_2 concentration affect Rubisco kinetics; (ii) to test whether current Rubiscos are optimally suited for the present conditions in the chloroplast of higher plants; (iii) to estimate the potential impact of climate change on the optimality of Rubisco kinetics and to identify targets of improvement. We argue that it is necessary to consider the tradeoff between specific activity and specificity to define rigorous criteria for engineering plants with *more efficient* Rubisco.

2. Methods and theory

2.1. Optimal Rubisco

According to the biochemical model of leaf photosynthesis [26], the net assimilation rate (A) is determined by the minimum of the RuBP-saturated (A_c) and RuBP-limited (A_j) CO_2 assimilation rates:

$$A = \min(A_c, A_j), \tag{1}$$

$$A_c = \frac{V_{cmax}(C_c - \Gamma^*)}{C_c + K_c(1 + O/K_o)} - R_d \tag{2}$$

$$A_j = \frac{(C_c - \Gamma^*)J/4}{C_c - 2\Gamma^*} - R_d \tag{3}$$

where V_{cmax} is the maximal carboxylation rate, C_c and O are the concentration of CO_2 and O_2 at the site of carboxylation in the chloroplast stroma, K_c is the Michaelis–Menten constant for CO_2 and K_o is that for O_2, Γ^* is the CO_2 compensation point in the absence of mitochondrial respiration, J is the CO_2-saturated electron transport rate of the thylakoid reactions which ultimately supply the necessary energy in the form of ATP and NADPH for the regeneration of RuBP, and R_d is the mitochondrial respiration in the light. The response of J to the photosynthetic photon flux density (PPFD) was calculated from the non-rectangular hyperbola proposed by Bernacchi et al. [27].

In Eq. (2), V_{cmax} was obtained from *in vitro* values as:

$$V_{cmax} = k^c_{cat} \cdot E \cdot Act \tag{4}$$

k^c_{cat} is the Rubisco maximum turnover rate of carboxylation, E is the total quantity of catalytic sites and Act is the percentage of activation of Rubisco sites, *i.e.* its carbamylation state.

Γ^* was obtained from the Rubisco specificity factor, $S_{c/o}$, as:

$$\Gamma^* = \frac{0.5O}{S_{c/o}} \tag{5}$$

Because the Rubisco kinetic parameters are interrelated [5,28], we can express Eqs. (2) and (3) as determined by a unique kinetic parameter, similar to the approach followed by Savir et al. [14]. k^c_{cat} is the most reported parameter, probably due to its relative ease of measurement. Thus, Eqs. (2) and (3) were reformulated by substituting Γ^*, K_c and K_o by the statistical functions relating them to k^c_{cat}. To obtain the relationships of Γ^*, K_c and K_o *vs.* k^c_{cat} at 25 °C we used the data compiled in Savir et al. [14], excluding the photosynthetic bacteria. The regression coefficients were highly significant ($P < 0.001$), with $r^2 > 0.75$ (Table 1S). Because the different kinetic parameters present distinct sensitivities to temperature [29], the functions relating k^c_{cat} with the remaining parameters are dependent on temperature. Hence, to interrelate the Rubisco kinetic parameters at temperatures other than 25 °C, values of k^c_{cat}, Γ^*, K_c and K_o were converted to the desired temperature using the equations described in Bernacchi et al. [29] for tobacco with the *in vitro* species-specific data measured at 25 °C as the reference (*i.e.*, the scaling constant c). Thereafter, regression analyses were again run between k^c_{cat} and Γ^*, K_c and K_o (Table 1S). The analysis therefore assumes that the Rubisco kinetic parameters of the species included in the present study presented the same temperature dependency as that for Rubisco from *Nicotiana tabacum*, and that the equations hold for the whole range of temperatures from 15 °C to 38 °C considered in the present study [30].

2.2. Optimization of Rubisco kinetics at varying temperature, and CO_2 and O_2 concentration

A first aim of the study was to model how temperature and $[CO_2]$ and $[O_2]$ affect Rubisco kinetics. For this, the optimal value for k^c_{cat} (*i.e.* the one maximizing the net CO_2 assimilation rate) was modeled at varying C_c and given values for J, R_d, E and Act by solving Eqs. (1)–(3) expressed in terms of k^c_{cat} as the unique parameter, using Mathematica 9 software (Wolfram Research, Champaign, IL, USA). This analysis was performed at three different temperatures (15, 25