Letter to the editor

How various factors influence the CO₂/O₂ specificity of ribulose-1,5-bisphosphate carboxylase/oxygenase

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Abstract

Temperature, activating metal ions, and amino-acid substitutions are known to influence the CO₂/O₂ specificity of the chloroplast enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase. However, an understanding of the physical basis for enzyme specificity has been elusive. We have shown that the temperature dependence of CO₂/O₂ specificity can be attributed to a difference between the free energies of activation for the carboxylation and oxygenation partial reactions. The reaction between the 2,3-enediolate of ribulose 1,5-bisphosphate and O₂ has a higher free energy of activation than the corresponding reaction of this substrate with CO₂. Thus, oxygenation is more responsive to temperature than carboxylation. We have proposed possible transition-state structures for the carboxylation and oxygenation partial reactions based upon the chemical natures of these two reactions within the active site. Electrostatic forces that stabilize the transition state of the carboxylation reaction will also inevitably stabilize the transition state of the oxygenation reaction, indicating that oxygenase activity may be unavoidable. Furthermore, the reduction in CO₂/O₂ specificity that is observed when activator Mg²⁺ is replaced by Mn²⁺ may be due to Mg²⁺ being more effective in neutralizing the negative charge of the carboxylation transition state, whereas Mn²⁺ is a transition-metal ion that can overcome the triplet character of O₂ to promote the oxygenation reaction.

Abbreviations: CABP – 2-carboxyarabinitol 1,5-bisphosphate; enol-RuBP – 2,3-enediolate of ribulose 1,5-bisphosphate; Kc – Km for CO₂; Ko – Km for O₂; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP – ribulose 1,5-bisphosphate; Vc – Vmax for carboxylation; Vo – Vmax for oxygenation

Introduction

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco, EC 4.1.1.39) is a bifunctional enzyme that catalyzes both the carboxylation and oxygenation of ribulose 1,5-bisphosphate (RuBP) in photosynthetic organisms (reviewed by Andrews and Lorimer 1987). The ratio of carboxylation to oxygenation rates can be related to the kinetic constants of the enzyme as:

\[ \frac{v_c}{v_o} = \frac{V_c}{V_o} \cdot \frac{K_o [CO_2]}{K_c [O_2]} \]  

where \( v_c \) and \( v_o \) are the velocities of carboxylation and oxygenation, \( V_c \) and \( V_o \) are \( V_{max} \) values for carboxylation and oxygenation, and \( K_c \) and \( K_o \) are \( K_m \) values for CO₂ and O₂ (Laing et al.


Thus, the $\text{CO}_2/O_2$ specificity factor, $\Omega = V_c K_c / V_o K_o$, is the kinetic constant that ultimately determines the relative rates of photosynthesis and photorespiration in plants. An increase in $\Omega$ would be expected to improve plant productivity by enhancing the photosynthetic rate (Ogren 1984).

To improve $\Omega$, one needs to understand how the structure of the Rubisco enzyme can determine this important kinetic parameter. It is known that $\Omega$ can be influenced by temperature (Jordan and Ogren 1984), activating metal ions (Jordan and Ogren 1981b), amino-acid substitutions (Chen et al. 1988, 1990, 1991, Chen and Spreitzer 1989), and chemical modification (Smith et al. 1990). Efforts to understand the influence of these factors on $\text{CO}_2/O_2$ specificity may serve as an appropriate starting point for ultimately improving the enzyme.

The kinetic determinants of Rubisco $\text{CO}_2/O_2$ specificity have been previously investigated by using the carboxylated six-carbon intermediate as a substrate for Rubisco enzymes that differ in their value for $\Omega$ (Pierce et al. 1986). It appears that the carboxylation or oxygenation of the 2,3-enediolate of RuBP (enol-RuBP) is irreversible. Thus, this point can be illustrated with respect to Eq. (1) as:

$$\frac{\text{CO}_2}{[\text{O}_2]} = \frac{v_c}{v_o} = \frac{k_c [E]_0 [\text{CO}_2]}{k_o [E]_0 [\text{O}_2]}$$  \hspace{1cm} (2)$$

where $k_c$ and $k_o$ are the rate constants for the partial reactions and $[E]_0$ is the concentration of the enzyme-enol-RuBP complex. By cancelling terms in Eq. (2), it becomes clear that $\Omega$ must also be equal to the ratio of the rate constants ($k_c / k_o$) for these two partial reactions (Fig. 1, Pierce et al. 1986). Based on transition-state theory (Fersht 1985), a rate constant ($k$) can be defined as:

$$k = \frac{kT}{h} e^{-\Delta G^*/RT}$$  \hspace{1cm} (3)$$

where $k$ is the Boltzmann constant, $h$ is Planck's constant, $R$ is the gas constant, $T$ is the absolute temperature, and $\Delta G^*$ is the free energy of activation. We previously showed that $k_c / k_o$ could be related to the carboxylation and oxygenation free energies of activation ($\Delta G^*_c$ and $\Delta G^*_o$) by the following equation (Chen and Spreitzer 1991):

$$\ln \frac{k_c}{k_o} = \frac{\Delta G^*_o - \Delta G^*_c}{RT}$$  \hspace{1cm} (4)$$

It is apparent from this equation that $k_c / k_o$ can be influenced by temperature (Chen and Spreitzer 1991), or, in a quite different way, by changes in $\Delta G^*_o - \Delta G^*_c$. Since $\text{CO}_2$ and $\text{O}_2$ do not bind to Rubisco (reviewed by Andrews and Lorimer 1987), and since enzyme-bound enol-