pH Dependence of Photosynthesis and Photorespiration in Soybean Leaf Cells

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JEROME C. SERVATIES
Department of Agronomy, University of Illinois, Urbana, Illinois 61801

WILLIAM L. OGREN
United States Department of Agriculture, Agriculture Research Service, Urbana, Illinois 61801

ABSTRACT

The effect of pH on the kinetics of photosynthesis, O₂ inhibition of photosynthesis, and photorespiration was examined with mesophyll cells isolated from soybean (Glycine max [L.] Merr.) leaves. At constant, substratating bicarbonate concentration (0.5 mm), O₂ inhibition of photosynthesis increased with increasing pH because high pH shifts the CO₂-bicarbonate equilibrium toward bicarbonate, thereby reducing the CO₂ concentration. At constant, substratating CO₂ concentrations, cell photorespiration decreased with increasing pH. This was indicated by decreases in the CO₂ compensation concentration, O₂ inhibition of photosynthesis, and glycine synthesis. Km(CO₂) values for isolated cell photosynthesis and in vitro ribulose-1,5-diphosphate carboxylase activity decreased with increasing pH, while the Ki(O₂) for both systems was similar at all pH values. The responses to pH of the corresponding kinetic constants of cell photosynthesis and in vitro RuDP carboxylase with respect to CO₂ and O₂ were identical. This provides additional evidence that the relative rates of photosynthesis and photorespiration in C₃ plants are determined by the kinetic properties of RuDP carboxylase.

The processes of photosynthesis and photorespiration are linked together by the carboxylase and oxygenase activities of RuDP² carboxylase, and the relative rates of these processes can be expressed in terms of the kinetic properties of RuDP carboxylase with respect to the two mutually competitive substrates CO₂ and O₂ (7, 8, 10). In this analysis, the rate of photorespiration relative to photosynthesis is given by the equation:

\[ \text{Photorespiration/photosynthesis} = \frac{t \cdot v_o}{v_e} = \frac{tV_oK_oO/K_oC}{V_eK_e} \]  
(1)

where \( v_o \) and \( v_e \) are the velocities of the oxygenase and carboxylase activities, respectively; \( t \) is a constant representing the stoichiometry between the amount of O₂ taken up in the oxygenase reaction to the amount of CO₂ released in photorespiration; \( V_o \) is the \( V_{max} \) of the oxygenase reaction; \( V_e \) is the \( V_{max} \) of the carboxylase reaction; \( K_o \) is the \( K_m(CO_2) \); \( K_e \) is the \( K_m(O_2) \); \( O \) is the O₂ concentration; and \( C \) is the CO₂ concentration. At standard atmospheric conditions, \( O \) and \( C \) are constant, being 21% O₂ and about 320 μL/L of CO₂. From activation energy measurements of oxygenation and carboxylation activities of the purified enzyme, \( V_e/V_o \) apparently is constant (8). Thus, the ratio of photorespiration to photosynthesis is governed by the relative values of \( K_o \) and \( K_e \).

The rate of photorespiration, relative to photosynthesis, increases with increasing temperature. Measurements on soybean leaves (8), isolated soybean leaf cells (15), and purified soybean RuDP carboxylase (8) indicated that increasing temperature increased \( K_o \) but had little effect on \( K_e \). Thus, the temperature dependence of photorespiration can be explained by the concepts embodied in equation 1. Another environmental parameter that may alter the relationship between photorespiration and photosynthesis is pH. In algae (11) and chloroplasts (5, 12), it was concluded that photorespiration increased at high pH values because increasing the pH increased the rate of synthesis of glycolate. This conclusion was supported by experiments with RuDP carboxylase indicating that the pH optimum for oxygenation was higher than that for carboxylation (1, 2). Bowes et al. (3), however, found that increasing the pH greatly decreased the \( K_m(CO_2) \) of RuDP carboxylase, and Nishimura et al. (9) found that the \( K_i(O_2) \) of spinach protoplast photosynthesis was similar at pH 7 and pH 8.5. According to equation 1, a decrease in \( K_o \) at constant \( K_e \) should reduce the ratio, \( K_o/K_e \), thereby reducing \( v_o/v_e \) and photorespiration. Because of the prior contradictory findings, the effect of pH on photorespiration was reexamined with soybean leaf cells, preparations which photospire (14, 15), yet, unlike leaves, can be assayed in aqueous solution. A preliminary report of the results reported here has been presented (13).

MATERIALS AND METHODS

Cells were isolated from mature leaves of soybean (Glycine max [L.] Merr. cv. Wayne) and assayed as described earlier (16), except that the buffer was 25 mM HEPES and 25 mM Tricine. Glycine accumulation was determined in the presence of 5 mM INH (14). Soybean RuDP carboxylase was partially purified and assayed as described by Keck and Ogren (7). The buffering capacity of the bicarbonate was compensated for by the addition of calculated amounts of HCl to retain the pH at the required level. CO₂ concentrations at pH 7.2, 7.8, and 8.8 were calculated from the amount of bicarbonate added to the assay vials and the dissociation constant of carbonic acid as given by Gibbons and Edsall (6). At pH 7.2 and 0.25 mM bicarbonate, 22% of the added bicarbonate was fixed during the cell photosynthesis assay, causing an 8% underestimation of the rate. A progressively smaller proportion of the bicarbonate was fixed at higher bicarbonate concentrations and higher pH values. Because this amount of bicarbonate depletion had only a slight effect on the \( K_m(CO_2) \) values, observed rather than corrected photosynthesis rates were used in Figure 3.

1 Present address: Department of Agronomy, University of Wisconsin, Madison, Wisconsin 53706.
2 Abbreviations: RuDP: ribulose 1,5-diphosphate; F: CO₂ compensation concentration; INH: iisonicotinic acid hydrazide.
RESULTS

In soybean cells at 0.5 mM bicarbonate, O2 inhibition of photosynthesis increased with increasing pH (Fig. 1), which is consistent with previous reports (5, 11, 12). Experiments of this kind, conducted at constant bicarbonate concentration, do not take into account the fact that the substrate for RuDP carboxylase is CO2 and not bicarbonate (4). As pH increases, the equilibrium between CO2 and bicarbonate shifts toward bicarbonate while the effect on O2 concentration is negligible. Thus, Figure 1 does not necessarily mean that O2 is intrinsically more inhibitory at high pH, but merely that high pH reduces the CO2 concentration so that CO2 competes much less favorably with O2 for RuDP. Indeed, the CO2 concentration in our assay mixtures (Fig. 1) was reduced from 470 μM at pH 5 to 0.7 μM at pH 9. In terms of equation 1, increasing pH increases the O/C ratio, thus increasing photorespiration and the susceptibility of the cell to O2 inhibition.

To determine the effect of O2 on photosynthesis at constant CO2, soybean cell photosynthesis was measured as a function of pH at several bicarbonate concentrations under 0% (N2) and 50% O2 (Fig. 2). Using the dissociation constant of carbonic

![Fig. 1. Effect of pH on soybean cell photosynthesis and O2 inhibition at constant bicarbonate concentration (0.5 mM). Reaction mixtures contained 10 μg of Chl in 1 ml and were terminated after 15-min illumination.](Image)

![Fig. 2. CO2 response curves of soybean cell photosynthesis. Reaction mixtures contained 7.5 μg of Chl in 1 ml and were terminated after 15-min illumination. Reactions were run in N2 (O—O) and 50% O2 (●—●).](Image)

![Fig. 3. Double reciprocal plots of CO2 response curves of soybean cell photosynthesis. Constructed from the data in Figure 2. For kinetic analysis of 50% O2 curves, F was subtracted from bicarbonate concentration before plotting.](Image)
The CO₂ concentration was calculated from the amount of bicarbonate added. The O₂ inhibition of photosynthesis was evident at all three pH values. At 50% O₂, the extrapolated intercept of the CO₂ response curves did not pass through the origin, but through a positive CO₂ value. This point is Γ, the CO₂ compensation concentration. The O₂ inhibition was quantitated by use of double reciprocal plots (Fig. 3). Oxygen was a competitive inhibitor of photosynthetic CO₂ fixation at all pH values.

The kinetic data derived from Figures 2 and 3 are summarized in Table I. The Km(CO₂) decreased from 41 μM CO₂ at pH 7.2 to 9 μM CO₂ at pH 8.8, while the Ki(O₂) was similar at all pH values. At 10 μM CO₂, O₂ inhibition of photosynthesis decreased from 63% at pH 7.2 to 37% at pH 8.8. The extrapolated Γ decreased from 130 μl/l of CO₂ at pH 7.2 to 35 μl/l of CO₂ at pH 8.8. The reduced O₂ inhibition and Γ at high pH indicate that photorespiration decreases with increasing pH because of an increased affinity of the cells for CO₂ at high pH.

Additional evidence on the effect of pH on photorespiration was obtained by allowing cells to photosynthesize in the presence of INH, a compound which inhibits the photorespiratory conversion of glycine to serine in soybean cells (14). At any given CO₂ concentration, glycine synthesis was greatest at pH 7.2 and least at pH 8.8 (Fig. 4). At 10 μM CO₂, for example, glycine accumulation decreased from 41% of the total carbon fixed at pH 7.2 to 23% at pH 8.8. These data demonstrate directly that the rate of photorespiration, relative to photosynthesis, is decreased by increasing pH.

### Table I. Kinetic parameters of soybean cell photosynthesis at pH 7.2, 7.8, and 8.8

<table>
<thead>
<tr>
<th>Parameter</th>
<th>pH 7.2</th>
<th>pH 7.8</th>
<th>pH 8.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Km(CO₂), μM</td>
<td>41</td>
<td>26</td>
<td>9</td>
</tr>
<tr>
<td>Ki(O₂), μM</td>
<td>780</td>
<td>650</td>
<td>660</td>
</tr>
<tr>
<td>Vmax</td>
<td>98</td>
<td>123</td>
<td>105</td>
</tr>
<tr>
<td>Photosynthesis rate²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 10 μM CO₂</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>N₂</td>
<td>19</td>
<td>34</td>
<td>57</td>
</tr>
<tr>
<td>50% O₂</td>
<td>7</td>
<td>18</td>
<td>36</td>
</tr>
<tr>
<td>O₂ inhibition, %</td>
<td>63</td>
<td>47</td>
<td>37</td>
</tr>
<tr>
<td>Γ, μl/l CO₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N₂</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50% O₂</td>
<td>130</td>
<td>70</td>
<td>35</td>
</tr>
</tbody>
</table>

¹Data from Figures 2 and 3. ²μmol CO₂/mg Chl-hr

Fig. 4. Effect of pH on glycine synthesis in soybean cells. Reaction mixtures contained 41 μg of Chl and 5 μM INH in 1 ml. After separation of products by column and paper chromatography (14), percent glycine was obtained by dividing dpm in glycine by total dpm fixed by the cells during 15-min illumination.

Fig. 5. Double reciprocal plots of CO₂ response curves of RuDP carboxylase in N₂ and 50% O₂. Reaction mixtures contained 30 μg of protein in 1 ml. Reactions were terminated after 3 min.
If the kinetic properties of RuDP carboxylase regulate the balance between photorespiration and photosynthesis, as formulated in equation 1 and elsewhere (7, 8, 10), then pH ought to alter the kinetics of this enzyme in a manner consistent with the observations made with cells (Table I). Soybean RuDP carboxylase activity was measured at 0% and 50% O₂ as a function of CO₂ concentration at pH 7.2, 7.8, and 8.8. Double reciprocal plots were constructed (Fig. 5) and the kinetic properties are summarized in Table II. O₂ is a competitive inhibitor of CO₂ incorporation at all pH values. As was found previously (3), increasing pH decreases the Km(CO₂). Ki(O₂) is not altered by pH. Thus, the ratio, Kₐ/Kₐ, decreases with increasing pH, and the enzyme is less sensitive to O₂ at high pH. This is reflected in the O₂ inhibition at 10 μM CO₂, which decreases from 44% at pH 7.2 to 29% at pH 8.8.

**DISCUSSION**

The results obtained with soybean cells indicate that the CO₂-bicarbonate equilibrium must be considered when determining the effect of pH on photosynthesis and photorespiration. If the two processes are measured at constant bicarbonate concentration, increasing pH decreases photorespiration and decreases photosynthesis because the CO₂ concentration decreases, allowing O₂ to compete more favorably for RuDP. But if constant CO₂ is maintained by altering the bicarbonate concentration, increasing pH decreases photorespiration and increases photosynthesis because of an increased affinity of the photosynthetic system for CO₂. Both of these situations are described by equation 1. At constant total CO₂ plus bicarbonate, CO₂ concentration decreases with increasing pH. Because O₂ concentration is unaffected by pH, the ratio of O/C increases so νv/νc, the ratio of photorespiration to photosynthesis, also increases. At constant CO₂ the ratio of O/C is constant at all pH values. But increasing pH decreases Kc while K₀ is unchanged, so the ratio of K₀/K₀ decreases and νv/νc decreases. The identical response of Kc and K₀ in isolated cells and in RuDP carboxylase activity in vitro provides additional evidence for the concept that the relative rates of photosynthesis and photorespiration are determined by the kinetic constants of RuDP carboxylase with respect to CO₂ and O₂ (7, 8, 10). The concentration of CO₂ and O₂ in leaves remains nearly constant because these two gases are in equilibrium with the atmosphere. Thus, in leaves, increasing pH will decrease photorespiration and increase photosynthesis.

Reduction or elimination of photorespiration may permit substantial increases in the productivity of C₃ crops (10, 17). Inhibition of the photorespiratory pathway, after the initial products of this pathway have been synthesized, does not increase net photosynthesis. Rather, inhibitors of photorespiratory glycylate metabolism increase the magnitude of O₂ inhibition (14). They probably prevent recycling of photorespiratory carbon back to the photosynthesis cycle, which leads to a depletion of the CO₂ acceptor, RuDP. Therefore, to reduce photorespiration it will be necessary to reduce the rate of glycylate synthesis. The data presented here indicate that glycylate synthesis can be reduced and CO₂ fixation increased by increasing pH at the site of RuDP carboxylase. Although increasing external pH from 7.8 to 8.8 reduced the Vmax of cell photosynthesis, it also increased the affinity of carboxylase for CO₂ such that the rate of CO₂ fixation at atmospheric CO₂ concentration (10 μM CO₂) was considerably increased (Table I) and the rate of photorespiration was greatly reduced (Fig. 4). Thus, there is considerable potential for increasing photosynthetic productivity by increasing chloroplast stromal pH. Although increasing stromal pH may have deleterious side effects, the potential benefits to crop productivity arising from control of photorespiration require that this possibility be thoroughly investigated.

**LITERATURE CITED**