Light-dependent Assimilation of Nitrite by Isolated Pea Chloroplasts

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ABSTRACT

Chloroplasts were prepared from peas (Pisum sativum) in glucose-phosphate medium. In the presence of Dl-glyceraldehyde, they catalyzed nitrite-dependent O2 evolution (mean of 13 preparations, 17.5 μ mole per mg chlorophyll per hour, SD 3.64). The optimum concentration of nitrite was 0.5 mM; 0.12 mM nitrite supported Vmax/2. The reaction was accompanied by the consumption of nitrite; 55 to 80% of the nitrite-N consumed was recovered as ammonia. In short experiments (less than 10 minutes) the O2 to nitrite ratio approached 1.5, but thereafter decreased. There was no nitrite-dependent O2 evolution with chloroplasts from plants grown without added nitrate but such chloroplasts could assimilate ammonia at about the usual rate. The results are consistent with the reduction of nitrite to ammonia involving nitrate-induced nitrite reductase and a reductant generated by the chloroplast electron transport chain.

In the presence of ADP, pyrophosphate, and MgCl2 the O2 to nitrite ratio was typically 0.5 to 0.6 and the recovery of nitrite-N as ammonia about 60%. Under these conditions, α-ketoglutarate increased the O2 to nitrite ratio (0.9–1.35) and the recovery of nitrite-N as ammonia decreased to 27%. These data and the results of nitrite plus ammonia addition experiments (with and without α-ketoglutarate) are attributed to incorporation of nitrite-N into glutamate via the chloroplast enzymes nitrite reductase, glutamine synthetase, and glutamate synthetase.

The enzymes glutamine synthetase and glutamate synthetase, which catalyze reactions now known to be basic for N assimilation, have been located in chloroplasts (7, 11, 13) and shown to be coupled to light-dependent electron transport. Thus, glutamate synthetase activity can be monitored by (glutamine plus α-ketoglutarate)-dependent O2 evolution (2). In the presence of the cofactors ADP, PPi, and Mg2+, pea chloroplasts also catalyze (ammonia plus α-ketoglutarate)-dependent O2 evolution which we attributed to assimilation of ammonia into glutamate via photo-synthetically coupled2 glutamine synthetase and glutamate synthetase (1).

Spinach chloroplasts in the light actively reduce nitrite (9, 10, 14); approximately 60 to 90% of the nitrite-N consumed is incorporated into amino-N (9, 10). Spinach chloroplasts also catalyze nitrite-dependent O2 evolution (10). According to current theory, nitrate-N is reduced to nitrite in the cytoplasm and further reduced to ammonia by nitrite reductase within the chloroplast. The ammonia so formed is incorporated via glutamine into glutamate in the chloroplast in reactions catalyzed by glutamine synthetase and glutamate synthase. In this event, nitrite-N should compete with glutamine-N and ammonia-N in the latter stages of the N assimilation pathway. We previously reported, however, that nitrite-dependent and (glutamine plus α-ketoglutarate)-dependent O2 evolution activities catalyzed by pea chloroplasts did not compete (2) implying that ammonia produced from nitrite was not assimilated by pea chloroplasts under the prevailing experimental conditions. We give a report in this paper of a study of light-dependent reduction and assimilation of nitrite by pea chloroplasts under conditions which (a) support and (b) do not support (ammonia plus α-ketoglutarate)-dependent O2 evolution.

MATERIALS AND METHODS

Seedlings of pea (Pisum sativum cv. Feltham First) were used. The methods for growing peas, measurement of Chl and chloroplast preparation (referred to in this paper as method 1) were as described previously (2). O2 evolution was measured polarographically and calibrated against air-saturated water at 25°C as before (2). Chloroplasts prepared by method 1 were incubated in sorbitol-HEPES medium (2) and were known to catalyze (glutamine plus α-ketoglutarate)-dependent O2 evolution and, in the presence of ADP, MgCl2 and PPi (ammonia plus α-ketoglutarate)-dependent O2 evolution (1, 2). Some aspects of nitrite reduction by method 1 chloroplasts were compared with chloroplasts used in a previous study of nitrite reduction (10). Method 2 chloroplasts were prepared essentially as described by Miflin (10) except that sorbitol was replaced by sucrose and with L-cysteine at 2 mM. Chloroplasts prepared by method 2 were incubated in sucrose-PPi medium (10). The composition of the extracting media were as follows: method 1: 0.33 mM glucose, 50 mM Na2HPO4, 50 mM KH2PO4, 5 mM MgCl2, 0.1% (w/v) NaCl, 0.2% (w/v) sodium ascorbate, 0.1% (w/v) BSA (type V) adjusted to pH 6.5 with KOH; method 2: 0.33 mM sucrose, 1 mM MgCl2, 2 mM EDTA, 2 mM L-cysteine, 10 mM Na2HPO4 adjusted to pH 7 with HCl. As summarized above, the incubating media were also different for the two methods (2, 10). Chloroplast intactness was determined by the ratio of the O2 evolution rates of shocked and unshocked chloroplasts using ferricyanide as electron acceptor (8).

Nitrite-dependent O2 evolution was measured at 25°C under two conditions. Condition 1 did not support (ammonia plus α-ketoglutarate)-dependent O2 evolution. Reaction mixtures contained 0.5 mM sodium nitrite, 10 mM DL-glyceraldehyde and chloroplasts (50–150 μg of Chl) in a volume of 2 ml. Condition 2 supported (ammonia plus α-ketoglutarate)-dependent O2 evolution (1). Incubation mixtures (2 ml) containing 4.5 mM ADP, 5 mM PPi, 10 mM MgCl2 and 10 mM DL-glyceraldehyde and chloroplasts (100–150 μg of Chl) were preincubated for 10 min with or without 2.5 mM α-ketoglutarate and the reaction initiated.

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4 Definition: the term "photosynthetically coupled" is used throughout this paper to refer to the generation of a reductant by photosystems I and II (e.g. reduced ferredoxin or NADPH) which is used as a substrate in the associated reaction.
Plant Physiol. Vol. 61, 1978  NITRITE ASSIMILATION BY PEA CHLOROPLASTS

with 0.5 mM sodium nitrite. (Ammonia plus α-ketoglutarate)-
dependent O₂ evolution and all other substrate-dependent O₂
evolution activities were measured at 25°C as before (1, 2).

The procedure for estimation of ammonia was based on a
method adopted for the determination of relatively small amounts
of free ammonia in samples containing amides (3). A very slow
stream of acid-washed N₂ gas was used to transfer 3 ml of saturated
sodium tetraborate (adjusted to pH 10 with NaOH) from a tall
tube (approximately 25 × 2.5 cm) into a similar one containing 2
to 4 ml of a mixture of sample with four times its volume of
ethanol. The gas was next led into 5 ml of 0.02 M H₂SO₄ in another
tube. Froth formation was controlled by addition of approximately
50 μl of octanol. Standard amounts of ammonia, estimated by the
method of Kaplan (6), were recovered quantitatively after 30-min
rapid gas flow.

Nitrite was measured by the method of Miflin (10). The decrease
in nitrite in reaction mixtures containing this substrate was taken
as a measure of nitrite reduction. Nitrate reductase was measured
in crude extracts (without an osmoticum) as described by Scholl
et al. (16).

RESULTS

O₂ Evolution and Nitrite Reduction for Condition 1. DL-Glyceraldehyde (10 mM) was usually added to reaction mixtures. It
inhibited endogenous O₂ evolution and enhanced the rate of
nitrite-dependent O₂ evolution (Fig. 1). In the absence of glyceraldehyde, the rate of nitrite-dependent O₂ evolution (which was
always measured after endogenous O₂ evolution ceased, Fig. 1)
gradually decreased. In the presence of glyceraldehyde, however,
the rate of nitrite-dependent O₂ evolution was constant for at least
5 min (Figs. 1 and 2). Provided measurements were taken during
this period the molar ratio of O₂ evolved to nitrite reduced
approached the theoretical value of 1.5 (Fig. 2). Molar ratios
substantially less than this value were found (in the presence of
glyceraldehyde) for some experiments (Table I). In general, low
ratios were associated with those experiments which either ex-
hibited a large decrease in the rate of O₂ evolution during the
incubation or were measured over a long time period. This is
shown by the data in Figure 3; the molar ratio decreased from
approximately 1.2 at 10 min to about 0.7 after 50 min. Certain of

![Figure 1](https://www.plantphysiol.org/content/61/2/693/F1.large.jpg)

**Fig. 1.** Effect of DL-glyceraldehyde and nitrite on O₂ evolution by method 1 chloroplasts for condition 1. The reaction mixtures (A and B) initially contained chloroplasts only; additions were made as shown. Reactions were timed from the moment of illumination. Values beside the curves represent rates of O₂ evolution in μmol mg of Chl⁻¹ hr⁻¹. Chl concentration, 50 μg ml⁻¹; chloroplast intactness, 74%; volume, 2 ml.

![Figure 2](https://www.plantphysiol.org/content/61/2/693/F2.large.jpg)

**Fig. 2.** Typical nitrite-dependent O₂ evolution curves for short term experiments and the associated nitrite reduction catalyzed by method 1 chloroplasts under condition 1. Double-tailed arrows show the time of illumination. Samples were removed for nitrite estimations immediately prior to illumination and again at the times indicated by single-tailed arrows. Other treatments were as shown. Appropriate details for each experiment (A to D) were as follows:

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Chl concentration (μg ml⁻¹)</th>
<th>Intactness (%)</th>
<th>Time of sampling for nitrite (min)</th>
<th>Nitrite consumed (nmol ml⁻¹)</th>
<th>O₂/nitrite ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>100</td>
<td>69</td>
<td>5.7</td>
<td>103</td>
<td>1.62</td>
</tr>
<tr>
<td>B</td>
<td>100</td>
<td>78</td>
<td>11.5</td>
<td>232</td>
<td>1.19</td>
</tr>
<tr>
<td>C</td>
<td>95.2</td>
<td>75</td>
<td>10.8</td>
<td>223</td>
<td>1.18</td>
</tr>
<tr>
<td>D</td>
<td>105.5</td>
<td>62</td>
<td>3.0 (a)</td>
<td>47</td>
<td>1.25</td>
</tr>
</tbody>
</table>

All reaction mixtures contained 0.5 mM nitrite and 10 mM DL-glyceraldehyde. Values beside the curves represent rate of O₂ evolution in μmol mg of Chl⁻¹ hr⁻¹.

The results in Table I show that glyceraldehyde also led to higher
rates of nitrite reduction and higher molar ratios. Nitrite-dependent
O₂ evolution ceased abruptly in the dark and was completely
inhibited by 1.7 μM DCMU (Fig. 2). Chloroplasts also failed to
function nitrite reduction under these conditions.

When the nitrite concentration was progressively increased, the
rate of O₂ evolution increased sharply with nitrite concentration
up to 0.5 mM (Vmax/2 = 0.12 mm) followed by a more gradual
decrease (Fig. 4). Osmotically shocked chloroplasts did not cata-
lize nitrite-dependent O₂ evolution either in the light or in the
dark.

We found no correlation of nitrite-dependent O₂ evolution rates
with the observed percentage intactness of the unshocked pre-
parations (62–85% intact). However, the initial nitrite-dependent O₂
evolution rates for method 1 chloroplasts (mean of 13 determina-
ations, 17.5 μmol mg of Chl⁻¹ hr⁻¹, SD 3.64) were correlated with
rates of ferricyanide-dependent O₂ evolution catalyzed by shocked
chloroplasts uncoupled with 5 mM ammonia (557.3 μmol mg of

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Table I. Estimates of the $O_2$ nitrite ratio by method 1 and method 2 chloroplasts incubated under condition 1.

Glyceraldehyde (10 mg), a standard component for condition 1, was omitted in some experiments.

Abbreviations: n.d., not determined; Gald, glyceraldehyde.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Chloroplast preparation method</th>
<th>Chloroplast intactness (%)</th>
<th>Additions</th>
<th>Pre-illumination time (min)</th>
<th>Illumination time (min)</th>
<th>Rate of nitrite consumption ($\mu$mol mg Chl$^{-1}$ hr$^{-1}$) O$_2$ nitrite ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n.d.</td>
<td>1</td>
<td>nil</td>
<td>11.2</td>
<td>22.0</td>
<td>5.7 0.79</td>
</tr>
<tr>
<td>2</td>
<td>n.d.</td>
<td>1</td>
<td>nil</td>
<td>11.6</td>
<td>13.3</td>
<td>4.4 1.16</td>
</tr>
<tr>
<td>3A</td>
<td>73</td>
<td>1</td>
<td>nil</td>
<td>8.8</td>
<td>7.5</td>
<td>8.8 1.09</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>Gald</td>
<td>nil</td>
<td>3.6</td>
<td>8.8</td>
<td>10.9 0.79</td>
</tr>
<tr>
<td>4A</td>
<td>74.5</td>
<td>1</td>
<td>nil</td>
<td>20.9</td>
<td>13.3</td>
<td>6.4 1.16</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>Gald</td>
<td>nil</td>
<td>11.1</td>
<td>11.4</td>
<td>1.07 1.16</td>
</tr>
<tr>
<td>5A</td>
<td>75.5</td>
<td>2</td>
<td>nil</td>
<td>22.8</td>
<td>11.2</td>
<td>2.8 0.59</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>Gald</td>
<td>nil</td>
<td>11.1</td>
<td>5.2</td>
<td>0.75 1.16</td>
</tr>
</tbody>
</table>

Fig. 3. Nitrite-dependent $O_2$ evolution (continuous curve) and the $O_2$ to nitrite ratio (□) in a long term experiment catalyzed by method 1 chloroplasts under condition 1. Chl concentration, 50 $\mu$g ml$^{-1}$; chloroplast intactness, 49%.

Fig. 4. Effect of nitrite concentration on the rate of $O_2$ evolution catalyzed by method 1 chloroplasts under condition 1.

$\text{Chl}^{-1}$ hr$^{-1}$, sd 76.2. Both the product-moment correlation coefficient and the Spearman rank correlation coefficient were significant at $P = 0.05$.

In some experiments, chloroplasts were supplied with limiting amounts of nitrite (typically 100 nmol ml$^{-1}$). Under these conditions $O_2$ evolution commenced in the usual way, but the rate gradually declined and finally ceased. When a further addition of nitrite was made, $O_2$ evolution recommenced. This sequence, like that for ($\alpha$-ketoglutarate plus glutamine)-dependent $O_2$ evolution (2) could be repeated several times. The molar ratio of $O_2$ evolved to nitrite supplied was calculated for each successive addition of nitrite for several experiments (Table II). Addition of $\alpha$-ketoglutarate (2.5 mM) did not affect the molar ratio for chloroplasts incubated under condition 1.

Chloroplasts prepared by method 1 or 2 did not catalyze $O_2$ evolution under condition 1 when nitrate was replaced by nitrate (0.05–1.6 mM) either in the absence or presence of 10 mM DL-glyceraldehyde. Addition of $\alpha$-ketoglutarate (2.5 mM) and/or azaserine (0.5 mM) did not affect the rates of nitrite-dependent $O_2$ evolution or nitrite reduction catalyzed by method 1 chloroplasts under condition 1.

Method 2 chloroplasts, incubated in sucrose-PPi medium in the absence of glyceraldehyde, consistently yielded higher rates of nitrite reduction in the presence of 10 mM $\alpha$-ketoglutarate and 10 mM pyruvate (Table III). However, neither $\alpha$-ketoglutarate nor pyruvate enhanced the rate of nitrite-dependent $O_2$ evolution (results not shown). Glyceraldehyde enhanced the rate of nitrite-dependent $O_2$ evolution by method 2 chloroplasts (Table I).

Oxygen Evolution and Nitrite Reduction for Condition 2. Method 1 chloroplasts catalyzed nitrite-dependent $O_2$ evolution under condition 2 at about 80% of the rate for condition 1. We employed the technique of measuring $O_2$ evolution for each addition of a limiting amount of nitrite to determine the molar ratio of $O_2$ evolved to nitrite supplied. In the absence of $\alpha$-ketoglutarate the ratio was typically 0.5 to 0.6 (Fig. 5B). In the presence of 2.5 mM $\alpha$-ketoglutarate, however, the ratio for the first addition of nitrite was 0.90 and thereafter increased with each successive addition (Fig. 5A). Furthermore, whereas the rate in the control decreased with each successive addition of nitrite, the rate of the reaction mixture containing $\alpha$-ketoglutarate (initially about 10% greater than the control) did not.

The effect of nitrite on the rate of $O_2$ evolution in the presence of 1 mM ammonia and 2.5 mM $\alpha$-ketoglutarate was also studied. Chloroplasts were preincubated in the presence of $\alpha$-ketoglutarate and $O_2$ evolution initiated with ammonia. Subsequent addition of nitrite enhanced the rate by 3.6 and 3.9 times in separate experi-
ments (Table IV). However, when the order of addition of ammonia and nitrite was reversed, the enhancement in the rate was much less than that predicted from the sum of the two independent activities when measured alone.

Ammonia Formation from Nitrite for Conditions 1 and 2. For condition 1, illuminated method 1 chloroplasts catalyzed the reduction of nitrite with the concomitant production of ammonia. The proportion of nitrite-N recovered as ammonia was typically 55 to 80% (Table V). For condition 2 in the absence of α-ketoglutarate, similar recoveries were obtained. In the presence of α-ketoglutarate, however, the proportion of nitrite-N recovered as ammonia was only 27% (Table V).

Nitrogen Assimilation by Method 1 Chloroplasts from Seedlings Grown With and Without Added Nitrate. An adequate supply of nitrate (10 mM) was routinely supplied to the seedlings to ensure establishment of the nitrite-reducing system. We investigated some reactions of N metabolism previously shown to be coupled to light-dependent electron transport in chloroplasts prepared from seedlings raised in Vermiculite with and without nitrate. The results (Table VI) show that the virtual absence of nitrite-dependent O₂ evolution in the chloroplasts of peas grown without nitrate was not accompanied by absence of activity with α-ketoglutarate plus ammonia, glutamine or aspartate, respectively. Crude extracts of peas grown without nitrate contained negligible nitrate reductase activity.

DISCUSSION

The results of the study of nitrite consumption, ammonia production, and O₂ evolution by method 1 chloroplasts for condition 1 in the presence of glyceraldehyde are in broad agreement with the operation of photosynthetically coupled nitrite reductase:

\[ \text{H}_2\text{O} + \text{HNO}_2 \xrightarrow{hv} \text{NH}_3 + 1/2\text{O}_2 \]  

(1)

The requirement for light and the sensitivity of the reaction to

DCMU (Fig. 1) demonstrate that light-dependent electron transport was involved. We presume that reduced ferredoxin, generated by the light reactions, serves as the reductant (4). For short-term experiments the O₂ to nitrite ratio approached the theoretical value of 1.5 (Fig. 1) and approximately 55 to 80% of the nitrite-N
Table V. Production of ammonia and O₂ in relation to 
nitrite consumed by method 1 chloroplasts under conditions 1 and 2

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Incubation condition</th>
<th>Chl concn.</th>
<th>Chloroplast Intactness</th>
<th>Period of illumination (min)</th>
<th>Rate of nitrite consumption</th>
<th>Nitrite produced</th>
<th>O₂ evolved</th>
<th>Nitrite consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(μg mL⁻¹)</td>
<td>(%)</td>
<td></td>
<td>(μmol mg Chl⁻¹ h⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>100</td>
<td>68.4</td>
<td>nil</td>
<td>0 - 3.5</td>
<td>21.0</td>
<td>0.66</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.5 - 7.43</td>
<td>13.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>100</td>
<td>76.4</td>
<td>nil</td>
<td>0 - 11.4</td>
<td>11.7</td>
<td>0.81</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.4 - 32.5</td>
<td>6.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>83</td>
<td>n.d.</td>
<td>nil</td>
<td>0 - 3.5</td>
<td>20.1</td>
<td>0.56</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.5 - 11.7</td>
<td>18.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4A</td>
<td>2</td>
<td>150</td>
<td>76.3</td>
<td>nil</td>
<td>0 - 21.3 (See Fig. 5B)</td>
<td>0.58</td>
<td>(See Fig. 5B)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>150</td>
<td>76.3</td>
<td>=-KG</td>
<td>0 - 18.9 (See Fig. 5A)</td>
<td>0.27</td>
<td>(See Fig. 5A)</td>
<td></td>
</tr>
</tbody>
</table>

Table VI. Activity of some photosynthetically coupled reactions of nitrogen 
metabolism in chloroplasts from pea seedlings grown with and without nitrate

Pea seedlings were grown with and without 10 mM KNO₃ in vermiculite for 11 days in a growth cabinet at 20°C and 12-h day length. Chloroplasts were prepared by method 1. The nitrate reductase activity 
associated with the soluble fraction was also measured. Abbreviation: =-KG, -=-ketoglutarate.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Activity</th>
<th>With Nitrate</th>
<th>Without Nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Chloroplasts</td>
<td>Intactness</td>
<td>68%</td>
<td>71%</td>
</tr>
<tr>
<td>Nitrite-dep. O₂ evolution</td>
<td>(Glutamate + -=KG)-dep. O₂ evolution</td>
<td>18.4 μmol mg Chl⁻¹ h⁻¹</td>
<td>0.3 μmol mg Chl⁻¹ h⁻¹</td>
</tr>
<tr>
<td>Soluble fraction</td>
<td>Nitrate reductase</td>
<td>3.6 μmol g FW⁻¹ h⁻¹</td>
<td>0 μmol g FW⁻¹ h⁻¹</td>
</tr>
<tr>
<td>2 Chloroplasts</td>
<td>Intactness</td>
<td>85%</td>
<td>71%</td>
</tr>
<tr>
<td>Nitrite-dep. O₂ evolution</td>
<td>(Aspartate + -=KG)-dep. O₂ evolution</td>
<td>8.0 μmol mg Chl⁻¹ h⁻¹</td>
<td>0 μmol mg Chl⁻¹ h⁻¹</td>
</tr>
<tr>
<td>Soluble fraction</td>
<td>Nitrate reductase</td>
<td>4.3 μmol g FW⁻¹ h⁻¹</td>
<td>0 μmol g FW⁻¹ h⁻¹</td>
</tr>
</tbody>
</table>

Table VII. Summary of the N-flux capacity of the component reactions of N-assimilation of isolated 
pea chloroplasts as determined from O₂ evolution

The rates of O₂ evolution are abstracted from this and previous reports (1,2). Abbreviations: =-KG, -=-ketoglutarate; Gln, glutamine.

<table>
<thead>
<tr>
<th>Substrate(s)</th>
<th>Rate of O₂ evolution (μmol mg Chl⁻¹ h⁻¹)</th>
<th>Theoretical N-flux per mol O₂</th>
<th>N-flux capacity (μg atom mg Chl⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrite</td>
<td>17.5</td>
<td>0.67</td>
<td>11.7</td>
</tr>
<tr>
<td>Gln + -=KG</td>
<td>10.6</td>
<td>2.0</td>
<td>21.2</td>
</tr>
<tr>
<td>NH₃ + -=KG</td>
<td>8.3</td>
<td>2.0</td>
<td>16.6</td>
</tr>
</tbody>
</table>

process other than the direct formation of reduced ferredoxin by 
the light reactions (also) operates for the synthesis of this reduc 
tant in the later stages of prolonged experiments. We presume 
that some nitrite-N is incorporated into organic-N since 20 to 45% of 
nitrite-N consumed was not recovered as ammonia. Although 
ammonia accumulated in chloroplasts under condition 1 this is 
unlikely to explain the decreased rates and lower O₂ to nitrite 
ratios in the latter stages of an incubation. The decreased rate 
of nitrite-dependent O₂ evolution as ammonia accumulated (Table 
V) suggests that under the prevailing experimental conditions the 
small amounts of ammonia produced did not cause uncoupling of 
the chloroplast electron transport chain.

The rates of light-dependent nitrite-reduction catalyzed by 
pea chloroplasts for condition 1 approximate those found for 
spinach (9, 10, 14) and maize (15), but the rate of nitrite-dependent 
O₂ evolution (17.5 μmol mg of Chl⁻¹ h⁻¹) was somewhat greater 
(10) presumably due to differences in species, chloroplast quality, 
and the use of glyceraldehyde. Method 1 pea chloroplasts, incubated 
under condition 1, appear to differ from spinach in that most of 
the nitrate-N consumed is recovered in ammonia. This implies 
that under condition 1 ammonia is not incorporated into 
amino-N to any great extent and would explain why α-ketoglutarate 
did not enhance the rate of nitrite reduction as reported for 
spinach (10) or nitrite-dependent O₂ evolution, or enhance the O₂ 
to nitrite ratio (Table II). However, the rate of nitrite reduction 
catalyzed by method 2 chloroplasts prepared and incubated in 
medium containing PPI was enhanced by α-ketoglutarate (Table 
III). Although the rates relative to those for method 1 chloroplasts 
are low, the results are consistent with the requirement for PPI for
the incorporation of ammonia into glutamine catalyzed by method 1 chloroplasts (1) as used in condition 2. Perhaps it is significant that experiments in which α-ketoglutarate has been shown to affect nitrite reduction (10) and incorporation of nitrite-N into amino-N (9) have been conducted in the presence of Pi buffer (10) or 0.5 mM Pi (9).

Plaut et al. (14) recently reported that pretreatment of intact and reconstituted chloroplasts systems with CO₂ and various intermediates of the CO₂ reduction pathway enhanced the rate of nitrite assimilation. They concluded that carbon cycle intermediates regulate, to some extent, nitrite reduction in vivo. Although we found that glyceraldehyde, an inhibitor of CO₂ assimilation (17) enhanced the rate of nitrite-dependent O₂ evolution and nitrite reduction (Table I), this apparent anomaly could be due to the enhanced availability of reductant (generated by the chloroplast electron transport chain) for nitrite reduction in the absence of any competing reactions of carbon metabolism. Alternatively, glyceraldehyde might interfere with the regulatory mechanisms for nitrite reduction.

Several features of the reactions catalyzed by chloroplasts incubated under conditions favorable for the operation of photosynthetically coupled glutamine synthetase and glutamate synthase (condition 2) differ from those for condition 1. Under condition 1 α-ketoglutarate did not affect the O₂ to nitrite ratio or the rates of nitrite-dependent O₂ evolution and nitrite reduction. For condition 2, however, α-ketoglutarate enhanced the O₂ to nitrite ratio, slightly enhanced the rate of O₂ evolution, and caused a decrease in the recovery of nitrite-N as ammonia. Taken collectively these results suggest that for condition 2 in the presence of α-ketoglutarate, nitrite-N is assimilated into glutamate via ammonia and glutamine, and involves the enzyme sequence nitrite reductase, glutamate synthetase, and glutamate synthase as follows:

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\[ \text{Glutamate} \rightarrow \alpha\text{-Ketoglutarate} \rightarrow \text{Glutamate} \]
\[ \text{NO}_2^- \rightarrow \text{NH}_3 \rightarrow \text{Glutamine} \]
\[ 3\text{H}_2\text{O} \rightarrow \text{ATP} \rightarrow \text{ADP + P}_i \rightarrow \text{H}_2\text{O} \rightarrow \alpha\text{-Ketoglutarate} \rightarrow \text{Glutamate} \]
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The involvement of the latter enzyme would enhance the theoretical O₂ to nitrite ratio to 2. The enhancement in the rates of O₂ evolution following the addition of nitrite to chloroplasts previously supplied with ammonia (Table IV) is in approximate agreement with the value of 4 predicted from theory on the assumption that no single reaction is rate-limiting (theory predicts for ammonia to glutamate, O₂:ammonia = 0.5; for nitrite to glutamate, O₂: nitrite = 2). Conversely, the small enhancement in the rate of O₂ evolution caused by ammonia when nitrite was supplied first (Table IV) suggests that the ammonia-dependent reactions were virtually saturated by ammonia produced from nitrite. The data summarized in Table VII indicate that the N-flux capacities of the component reactions of N-assimilation in chloroplasts as judged by O₂ evolution are of a similar order of magnitude, but that nitrite reductase is marginally rate-limiting. The data in Table IV are consistent with this possibility.

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LITERATURE CITED


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