Effect of CO₂ on Photophosphorylation in vivo as Revealed by the Light-dependent Cl⁻ Uptake in Elodea densa

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The light-dependent Cl⁻ uptake in Elodea densa exhibits dual DCMU inhibition curves dependent on the presence and absence of CO₂. This suggests that cyclic photophosphorylation (energy surce in the absence of CO₂) is also inhibited by higher concentrations of DCMU in vivo. Further, at low DCMU concentrations the Cl⁻ uptake in the presence of CO₂ is much smaller than would be expected if cyclic photophosphorylation were operative. This points to a parallel inhibition of non-cyclic and cyclic photophosphorylations by low concentrations of DCMU in the presence of CO₂. The apparent inhibition of cyclic photophosphorylation at low concentrations of DCMU can be reversed by withdrawal of CO₂. It is concluded that CO₂ may be important in the regulation of cyclic and non-cyclic photophosphorylations in vivo.

The occurrence in vivo of cyclic photophosphorylation besides the non-cyclic type is documented in several organisms mostly by the existence of a Cl⁻ uptake manifold as since light enhances the Cl⁻ uptake in the absence of CO₂. Cl⁻ uptake by photophosphorylation. However, little is known about the relative amounts of both types of photophosphorylation inside the cell and their mutual regulation. In a previous paper the light-dependent Cl⁻ uptake in Elodea densa during 30—40 min was shown to be supported by photophosphorylation. Cl⁻ uptake in Elodea seems to be a useful test reaction for non-cyclic and cyclic photophosphorylation in vivo since light enhances the Cl⁻ uptake manifold as compared to darkness under conditions in which non-cyclic and cyclic or only cyclic photophosphorylations are possible.

The present report gives evidence that the light-dependent Cl⁻ uptake in air exhibits different DCMU inhibition curves dependent on the presence and absence of CO₂. Further it is suggested that CO₂ may play an important part in the regulation of cyclic and non-cyclic photophosphorylations in vivo as proposed by Arnon. The results presented here lend further support to the view that ATP synthesis rather than photosynthetic electron transport sustains the active Cl⁻ transport in the light in Elodea contrary to the events in Nitella.

Material and methods

Elodea densa was grown in an artificial growth medium and illuminated in a 14/10 hours light and dark cycle. The growth medium contained 10⁻⁵ M Cl⁻ ions.

Cl⁻ uptake. Leaves of Elodea were detached and kept overnight in the dark in an inactive buffered solution corresponding to the reaction mixture but containing 10⁻⁵ M Cl⁻ ions. In the same (renewed) solution the leaves were pretreated for 40 min under the experimental conditions (temperature, light/dark, aeration). The uptake of Cl⁻ was started by exposure of the leaves to 10 ml reaction mixture containing in moles/l: Cl⁻: 2 x 10⁻⁴; Na⁺: 7.56 x 10⁻⁴; K⁺: 1.84 x 10⁻⁴; Ca²⁺: 1 x 10⁻⁴; Mg²⁺: 1 x 10⁻⁵; SO₄²⁻: 1.4 x 10⁻⁴; H₂PO₄⁻: 1 x 10⁻⁴; TRIS: 3.3 x 10⁻⁴; phthalate: 6.7 x 10⁻⁴; and ³⁵Cl⁻ at a specific activity of 22 µCi/m mole Cl⁻. The pH was 5.2. The temperature was maintained at 25 °C.

The solution was aerated and stirred with the gas mixtures as specified in the legends of figs. 1 and 2. The uptake of ³⁵Cl⁻ was terminated by thorough washing of the leaves with 5 x 10⁻⁴ M NaCl solution. The leaves were fixed to adhesive tape on planchets, dried, and counted with a thin window methane flow counter. The leaf area was determined by weighing. DCMU was added in methanol solution yielding a final concentration of 1% methanol that was also present in the controls. The photosynthetic O₂-production was measured polarographically. For details see l.c.⁶.

Material and methods

2. W. SIMONIS, Ber. dtsch. bot. Ges. 77, (5) [1964].
6. DCMU = 3-(3,4-dichlorophenyl) 1,1-dimethyleurea.
7. W. D. JESCHKE, Planta 67, 6 [1965].
Results and discussion

1. DCMU action on non-cyclic photophosphorylation. Fig. 1 presents the concentration curves of DCMU action on Cl\textsuperscript{-} uptake in Elodea leaves in the light and in darkness. Curve A, measured in air + 3% CO\textsubscript{2}, strongly resembles the inhibition curve of O\textsubscript{2}-production in Elodea\textsuperscript{6} as follows also from the similar concentrations of half inhibition (table 1). It can be concluded that in the presence of abundant CO\textsubscript{2} the active Cl\textsuperscript{-} uptake is supported energetically to a great extent by non-cyclic photophosphorylation. This finding seems important inasmuch as it implies that ATP produced by non-cyclic photophosphorylation is contributed to a pool of ATP that is also available for processes different from CO\textsubscript{2}-assimilation.

The uninhibited ca. 30% of Cl\textsuperscript{-} uptake (curve A) may be sustained either by cyclic photophosphorylation or by oxidative phosphorylation, since the depression of oxidative phosphorylation in the light\textsuperscript{2} does not necessarily remain, if photosynthesis is inhibited by DCMU.

2. DCMU inhibition of oxidative phosphorylation and cyclic photophosphorylation. As is seen from fig. 1, DCMU at high concentrations also affects the Cl\textsuperscript{-} uptake in the dark (curve C), probably by an unspecific action on oxidative phosphorylation that may be compared to the effects of DCMU in Ankistrodesmus\textsuperscript{7} and in Phormidium\textsuperscript{8}.

<table>
<thead>
<tr>
<th>Reactions and conditions</th>
<th>A</th>
<th>B</th>
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<tbody>
<tr>
<td></td>
<td>C\textsubscript{1}50 Cl\textsuperscript{-}uptake</td>
<td>[DCMU] [mole/l]</td>
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<tr>
<td>O\textsubscript{2}-production</td>
<td>light, 4000 Lux</td>
<td>1.7 \times 10\textsuperscript{-7}</td>
</tr>
<tr>
<td>Cl\textsuperscript{-} uptake, air, 3% CO\textsubscript{2}, light, 4000 Lux</td>
<td>2.5 \times 10\textsuperscript{-7}</td>
<td>6.4 (23)</td>
</tr>
<tr>
<td>Cl\textsuperscript{-} uptake, air, no CO\textsubscript{2}, light, 4000 Lux</td>
<td>1.1 \times 10\textsuperscript{-6}</td>
<td>7.5 (24)</td>
</tr>
<tr>
<td>Cl\textsuperscript{-} uptake, N\textsubscript{2}, no CO\textsubscript{2}, light, 4000 Lux</td>
<td>1.5 \times 10\textsuperscript{-6}</td>
<td></td>
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<tr>
<td>Cl\textsuperscript{-} uptake, air (0.03% CO\textsubscript{2}) darkness</td>
<td>1.4 \times 10\textsuperscript{-4}</td>
<td>1.6 (8)</td>
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Table 1. A. DCMU concentrations (C\textsubscript{1} 50) effecting 50% inhibition in several reactions of Elodea leaves; the figures were obtained as points of inflection of the inhibition curves. B. Absolute values of Cl\textsuperscript{-} uptake (influx) of Elodea leaves in 2 \times 10\textsuperscript{-4} M Cl\textsuperscript{-} solution. Averages of (n) determinations.

However, in the light a second inhibition curve of DCMU action can be obtained if the Cl\textsuperscript{-} uptake is measured in the absence of CO\textsubscript{2} in air (fig. 1, curve B) or in N\textsubscript{2}\textsuperscript{6}. Under these condition only cyclic (N\textsubscript{2}) or cyclic and oxidative phosphorylations (air without CO\textsubscript{2}) are possible. Since oxidative phosphorylation is not influenced except at much higher concentrations (fig. 1) it seems evident that in vivo also cyclic photophosphorylation is inhibited by DCMU as was also found in Chlorella\textsuperscript{4}. The relative sensitivities of Cl\textsuperscript{-} uptake in the presence (non-cyclic photophosphorylation) and in the absence of CO\textsubscript{2} (cyclic photophosphorylation) differ only by a factor 10 in DCMU concentration (fig. 1, table 1).

In isolated chloroplasts DCMU is without effect on cyclic photophosphorylation according to ARNON\textsuperscript{12} and to TREBST\textsuperscript{13}. On the other hand, ASAIH and JAGENDORF\textsuperscript{14} suggest a second site of DCMU action active in cyclic photophosphorylation. It is possible, however, that cyclic photophosphorylations as measured in vitro with the unphysiological phenazine methosulfate (PMS) or with large concentrations of ferredoxin\textsuperscript{15} as cofactors do not give a true picture of the events in vivo\textsuperscript{16}. Thus the affinity of DCMU to the PMS-mediated electron path may be smaller than to the cyclic electron flow in vivo. Also

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it is possible on the grounds of mass action that the high concentrations of ferredoxin as used in vitro require a greatly increased DCMU concentration for an inhibition.

3. Suppression of cyclic photophosphorylation at low DCMU concentrations in the presence of CO₂. The absolute values of CI⁰ uptake in the presence and in the absence of CO₂ are almost the same¹⁷ (table 1). Thus the inhibited values in fig. 1 can be directly compared and it follows that at 5 × 10⁻⁷ M DCMU the CI⁰ uptake in the absence of CO₂ (cyclic photophosphorylation) is considerably higher than in the presence of CO₂ (fig. 1). Under the provision that CI⁰ uptake is supported by photophosphorylation this implies that uninhibited cyclic photophosphorylation could sustain a substantially higher CI⁰ uptake than is actually found after inhibition by DCMU in the presence of abundant CO₂. Apparently at the low concentration (5 × 10⁻⁷ M) of DCMU not only non-cyclic but also cyclic photophosphorylation is greatly decreased in the presence of abundant CO₂. At least there is no compensation by cyclic photophosphorylation for the non-cyclic type after inhibition of the latter by DCMU — as was also postulated for Chlorella by Gould and Bassham¹⁸ for other reasons.

Apparently quite different situations obtain in vivo if the non-cyclic electron transport is suppressed at the reductive end of the electron chain (by withdrawal of CO₂) or at the oxidative end (by DCMU) provided that CO₂ is present. In the first case cyclic photophosphorylation still is possible and probably enhanced, in the second case it seems greatly to be suppressed too.

According to Tagawa et al. and to Arnon⁸, ⁹ the regulation in the cell between non-cyclic and cyclic photophosphorylation could be effected by the reduction state of NADP. On the basis of this scheme the results of fig. 1 can be explained: In case the noncyclic electron transport is inhibited by lack of CO₂, ferredoxin and NADP are not oxidized by CO₂. In the light they are continuously maintained in a reduced state. Thus an excess of electrons can flow back to some intermediate of the electron chain and thus permit a cyclic electron transport. If on the other hand the non-cyclic electron transport is inhibited at the site of oxygen evolution by DCMU in the presence of CO₂, then the few electrons arriving at ferredoxin or NADP will be consumed by the reduction of CO₂ leaving NADP in an oxidized state and an electron gap the site of ferredoxin or the substance Z¹⁹ of light reaction I. Thus cyclic electron transport cannot proceed to a great extent although it is not specifically inhibited by DCMU at this low concentration.

Starting from this explanation it can be predicted that the apparent inhibition of cyclic photophosphorylation at 5 × 10⁻⁷ M DCMU in the presence of CO₂ should be abolished if only after the inhibition CO₂ is removed.

The results of an appropriate experiment are shown in fig. 2. Elodea leaves were first treated with DCMU in the presence of 3% CO₂ resulting in an inhibited CI⁰ uptake. After 40 min in a part of the experimental vessels the gassing was changed afterwards to air without CO₂, the others still getting air + CO₂. CI⁰ uptake was measured for a second 40 min period in the presence of the same DCMU concentration.

The somewhat lower value in the presence of CO₂ points to a competition between CO₂ assimilation and CI⁰ uptake probably for ATP. At low light intensities CO₂ considerably inhibits the CI⁰ uptake in the light.

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more than twice as large as in the parallels with CO₂ although both samples still are incubated with $5 \times 10^{-7}$ M DCMU.

We interpret this result as proof of a resumption of cyclic photophosphorylation which now is possible again as electrons arriving at ferredoxin are no longer drained away by reduction of NADP or CO₂.

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Enzyme der Polynucleotid-Synthese in pflanzlichen Organismen

II. Isolierung, Reinigung und Charakterisierung der RNA-Polymerase aus der Blaualge

Anacystis nidulans

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The isolation and purification of RNA polymerase from the blue-green alga Anacystis nidulans is described. The method consists in an initial fractional centrifugation which yields 60—70% of the enzyme associated with a DNA-rich fraction. It is subjected to column chromatography (DEAE-cel- lulose) and gel filtration (Sephadex G-200) to obtain a 110—120 fold increase in specific activity over the crude extract. The preparation is free from polynucleotide phosphorylase, kinases, phosphatases and nucleic acids. The enzyme requires DNA, a primer, as well as Mg²⁺, Mn²⁺ and the 4 ribonucleoside triphosphates for the net synthesis of polyribonucleotides. Salmon sperm DNA and native DNA from Chlorella pyrenoidosa can replace Anacystis DNA as primer. The properties of the product, however, depend to a certain extent on the primer DNA used as revealed by column chromatography of the former on methylated serum albumin. After isolation by this method, the synthesized polyribonucleotides were found to be partially stable against the action of RNase. This finding suggests a possible association of the newly synthesized RNA with the primer DNA in form of a stable complex which may function as an intermediate of the DNA directed RNA synthesis.


1 G. RICHTER, Biochim. biophysica Acta [Amsterdam] 72, 342 [1963].