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EFFECT OF OZONE ON PHOTOSYSTEM II IN *SPINACIA OLERACEA* CHLOROPLASTS

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Abstract—Spinach plants were treated with 0·35 ppm ozone and the photochemical activity of photosystem II determined after separation from the isolated chloroplasts using digitonin. Ozone impaired the activity of the system and also reduced the amount of β-carotene in the chloroplasts.

INTRODUCTION

OZONE (O_3), the principal oxidant in smog, decreases the number of chloroplast ribosomes more than the number of cytoplasmic ribosomes.[1] Electron-microscopic examination of pinto bean leaves exposed to O_3 showed that the chloroplast stroma change before other subcellular components.[2] Light also alters the physiological responses of plants to ozone.[3] These investigations showed a close association between the action of O_3 and light-dependent chloroplasts. Light mediated reactions occur in chloroplasts at two sites—photosystems I and II. The photosynthetic pigments and electron carriers associated with O_2 evolution are known collectively as photosystem II, whereas photosystem I consists of pigments associated with NADP reduction. Both pigment systems contain chlorophyll *a*, chlorophyll *b* and the major carotenoids, but in different proportions. Chlorophyll *b* is concentrated in photosystem II and photosystem I has a higher content of β-carotene.[4] Digitonin, a nonionic detergent, will disperse chloroplasts into fragments; the larger fragments are enriched in photosystem II, whereas the smaller fragments have the properties of photosystem I.[4]

In the present study, we have investigated the influence of O_3 on the photochemical capacity of photosystem II and on the ratio of chlorophyll to β-carotene in the chloroplasts of spinach.

RESULTS AND DISCUSSION

The rate of photoreduction of 2,3,6-trichloroindophenol (TCIP), determined from the decrease in absorbancy at 620 nm/45 sec, was found to be linearly related to the

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[2] THOMPSON, W. W. (1966) *Can. J. Botany* **44**, 1977.
[3] TAYLOR, O. C., DUGGER, W. M., CARDIFF, E. A. and DARLEY, E. F. (1961) *Nature* **192**, 814.
[4] BOARDMAN, N. K. and ANDERSON, J. M. (1964) *Nature* **203**, 166.

chlorophyll concentration of the photosystem II pigments of spinach. The reaction rate (33·4 units) was very close to that previously reported for spinach.[5] The results also indicated that the light intensity was saturated under the experimental conditions used.

To determine the influence of O_3 on the photochemical activity of photosystem II, plants were exposed to 0·35 ppm O_3 for 50, 60, 70, and 80 min as described in the Experimental. The leaves were harvested immediately after treatment in an effort to determine the reaction rate before visible leaf symptoms developed. Such symptoms were observed about 12 hr after the treatment by 0·35 ppm O_3 for 70 min. Ozone curtails the activity of the Hill reaction, but the rate was never inhibited more than about 45% of the control level, regardless of the length of the treatment. Inhibition was maximal within about 10 min after O_3 began to affect the activity (after 60 min). A similar response has also been observed in the chloroplast ribosomes of pinto bean leaves exposed to O_3.[1] These observations indicate that chloroplasts are an O_3 sensitive site in plants and it is postulated that O_3 restricts the capacity of photosystem II to accept electrons from OH ions. Such a restriction would also coincide with a decrease in O_2 evolution, one of the reactions involved in the photosynthetic process.

Previous work, however, on bean plants treated with O_3,[6] has shown that detectable reductions in the apparent rate of photosynthesis only occurred in leaves showing visible signs of tissue damage. The results indicated that the reduced rate of photosynthesis was due to the loss of chloroplasts caused by tissue damage, since O_3 virtually destroyed the injured leaf areas.[6] This previous report is in contrast with our results, which demonstrate a direct effect of O_3 on the Hill reaction.

Ozone also modified the ratio of chlorophyll to β-carotene in spinach chloroplasts. A mean value of 88·8 μg of β-carotene/4·8 mg of chlorophyll was found in the control plants and a mean value of 54·1 μg of β-carotene for the same amount of chlorophyll in the O_3 treated plants. Stanier[7] postulated that carotenoids protect chlorophyll from photo-oxidation. Ozone probably oxidizes β-carotene into one of the other carotenoids. In addition, O_3 may attack double bonds of unsaturated fatty acids associated with the chloroplasts.

Our findings in this communication of the effects of O_3 on the site of the Hill reaction (photosystem II) contribute to the previous observations that chloroplasts are closely linked with the action of O_3 in plant systems.[1, 3]

EXPERIMENTAL

Spinach plants (*Spinacia oleracea* L.) were grown and treated with O_3 as previously described,[1] except that the light intensity was 20 K lx. The chloroplasts were then isolated,[5] fragmented, and separated into various sizes of particles according to the procedures described by Boardman and Anderson:[4] Photosystem II was then identified and isolated.[4] The photochemical capcity of photosystem II was determined by measuring the level of photoreduction of TCIP.[5] The reaction mixture contained the suspension of chloroplast fragments equivalent of 8 μg chlorophyll, 72 μmol TCIP, and 150 μmol of Tris-HCl buffer at pH 7·2 in a total vol. of 3 ml. The reaction rate was determined by measuring the absorbancy (A) before and after illumination (18000 lx) for 45 sec at room temp. The unit of activity from this measurement was defined as change in A at 620 nm/g chlorophyll. The total amount of chlorophyll was extracted with MeOH and its content estimated by the equation described by Vishniac.[8]

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The β-carotene content of the sedimented chloroplast fragments was determined by a method for green-leaf material[9] with minor modifications. Chloroplast fragments (at 10000 *g*) were isolated and suspended in the grinding buffer. Replicate suspensions of chloroplast fragments, each of which contained 4·8 mg of chlorophyll, were prepared and sedimented by centrifugation. β-Carotene was extracted with Me_2CO–petrol. (1 : 1) containing 0·1% quinol. This process was continued until no more color could be extracted. Most of the Me_2CO was removed from sample extracts before loading on an alumina–Na_2SO_4 column by extracting with *ca* 1·5 l. H_2O. The trailing edge was fairly clear while the sample was eluted through the column, which indicated that the adsorbent would not cause isomerization. The yields from replicate samples varied not more than 6%. The amount of β-carotene was determined by relating the *A* at 450 nm to that of a standard curve for pure β-carotene.

[9] *The determination of carotene in green-leaf materials*—I. Fresh grass. in *Analytical Methods Committee, Report prepared by the Carotene Panel of the Sub-committee on Vitamin estimations*, (1950) *Analyst* **75**, 568–573.