Plant Physiol. (1976) 57, 308-309

The Occurrence of Peroxide in a Perennial Plant, *Populus gelrica*[1]

Received for publication August 6, 1975 and in revised form September 17, 1975

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ABSTRACT

A large amount of peroxide was found in twigs of poplar, *Populus gelrica*, which was grown in the field under natural conditions. The peroxide found in xylem and living bark was about 1.2 and 0.5 μmoles per gram dry weight sample, respectively, and served as a substrate both for catalase and cytochrome *c* peroxidase.

Patterson and Myers (5) described in *in vivo* formation of peroxide in a photosynthetic system using *Anacytis nidulans* and recently, Asada *et al.* (2) have shown the *in vitro* photosynthetic formation of peroxide and superoxide radical in chloroplasts. Although the amount of the peroxide found in the above reports was very small, these reports verified the earlier studies on the occurrence of peroxide in plants by Mehler (4). During the course of our experiments to determine a temporal control of the activities of glucose-6-P and 6-P-gluconate dehydrogenases in poplar tree (8, 9), a large amount of extractable peroxide was found in twigs of poplar, *Populus gelrica*, as described in this paper.

MATERIALS AND METHODS

Poplar twigs (*Populus gelrica*) were sampled from trees growing in the field and extracts for the analysis were prepared immediately. To assay "total peroxide," 1 g of poplar xylem was homogenized in the cold in the presence of 3.5 ml of cold 5% trichloroacetic acid (or perchloric acid) and 1 g of sea sand for 5 min. The slurry was pressed through a sheet of gauze about 7 cm. sq. and the resultant extract was centrifuged at 14,000*g* for 5 min. Immediately after the centrifugation, "total peroxide" in the supernatant was analyzed by the following ferrithiocyanate method (13).

The test tubes contained the extract equivalent of 0.05 g dry weight sample in a volume of 1.60 ml. To this solution was added 0.4 ml of 50% trichloroacetic acid, 0.4 ml of 10 mM ferrous ammonium sulfate, and color developed by the addition of 0.2 ml of 2.5 M potassium thiocyanate was read at 480 nm. The "total peroxide" used in the experiment of Cyt *c* peroxidase was extracted by homogenization of 3 g of wet xylem in the presence of 6 ml of cold 3% perchloric aicd and 3 g of sea sand for 5 min. The supernatant after centrifugation at 14,000*g* was neutralized with 4 N KOH and the neutralized extract, after removal of potassium perchlorate, contained about 240 nmoles of peroxide/ml as assayed by the ferrithiocyanate method (13). Catalase was a product of Sigma (C-100). Cyt *c* peroxidase was purified from commercial baker's yeast by the procedure of Yonetani and Ray (15) and the ratio of the absorbance at 408 nm to 280 nm of the present preparation was 0.405. The activity of the peroxidase as expressed by an initial absorbance decrease at 550 nm/min·μl of the enzyme in 1 ml (10 mm path length) was 1.2.

RESULTS AND DISCUSSION

A large amount of peroxide was present in poplar xylem. By homogenization of poplar xylem and living bark in the presence of 5% trichloroacetic acid or 3% perchloric acid, the peroxide could easily be extracted, while homogenization in the presence of 0.3 N HCl resulted in a lowered recovery of the peroxide. Sixty-three nanomoles of ferrous sulfate were oxidized by the extract equivalent to 50 mg dry weight xylem which was sampled from wintering 1-year-old twigs. The trichloroacetic acid extract of the xylem was neutralized with 4N KOH and 0.1 μg of catalase was added to the solution. Most of the "total peroxide" (57 out of 63 nmoles) was decomposed by the addition of catalase and about 10% (6 nmoles) of materials giving the color reaction remained unchanged after incubation for 5 min at room temperatures. Fifty-five nanomoles of an authentic H_2O_2 were completely decomposed by the addition of 0.1 μg of catalase under the same conditions.

Another experiment was done using Cyt *c* peroxidase, which is known to form a complex with peroxide with an absorption maximum at 419 nm (15). Cyt *c* peroxidase with an absorption maximum at 408 nm, was converted by the addition of the peroxide to complex II with an absorption maximum at 419 nm (Fig. 1). The peak positions of these spectra are in a good agreement with those reported for the enzyme by Yonetani and Ray (15). Further, no complex II formation could be observed after treatment of the neutralized extract with catalase (Fig. 1, line 4), nor any absorption could be seen in the solution of neutralized extract alone (line 3).

These results provide strong evidence for the presence of peroxide in the extract of poplar twigs. Trichloroacetic acid extracts of 10 samples of wintering twigs were analyzed for the "total peroxide" and the levels were found to reach an equivalent of about 1.2 ± 0.2 μmoles (in xylem) and 0.5 ± 0.2 μmole (in bark) of H_2O_2 per g dry weight sample. Since the wintering poplar twigs contain about 50% of water in weight, the peroxide reached a level of millimolar concentration, assuming that the peroxide existed uniformly in the cells. However, since the peroxide is highly toxic and would be localized in a particular compartment inside the cells or in the tissues, the actual level *in situ* would probably be much higher than the above concentration. An indentification of the molecular species of the peroxide as well as a biological significance in poplar tree remain to be determined. However, in plants, peroxide is definitely related to ethylene formation (12, 1). Also, peroxidative reactions are involved, *in vitro*, in lignin (11) and flavonoid (6) syntheses and in IAA metabolism (14). The findings of Rathmell *et al.* (7) in which they reported that the specific activity of peroxidase was 100-fold higher in the intercellular fluid than in the remaining cellular fraction was a point of great interest, since this higher specific activity of the enzyme suggests that a series of peroxidative reactions are taking place in the compartment. Also, perox-

[1] Contribution No. 1668 from the Institute of Low Temperature Science, Hokkaido University, Sapporo, Japan.

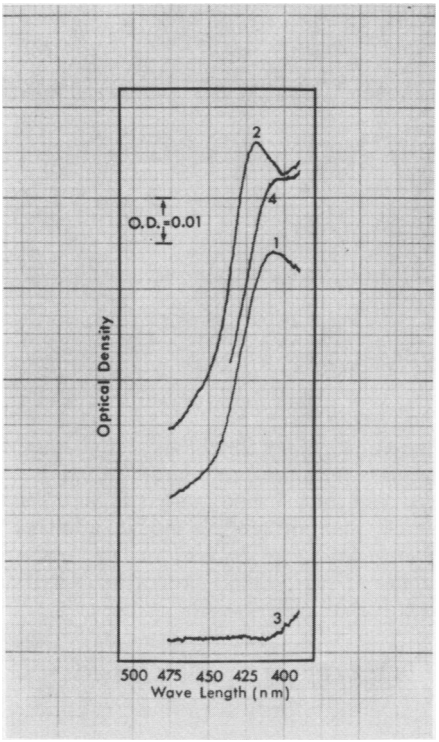

FIG. 1. Complex II formation of Cyt *c* peroxidase by the addition of the xylem peroxide. The spectrum of a solution containing 25 μmoles of potassium phosphate buffer of pH 6 and 0.9 ml of the Cyt *c* peroxidase (total activity; A_{550} = 144/min. cm) in a volume of 1.35 ml was first taken and is shown in line 1. To the solution was then added 0.15 ml of the xylem "total peroxide" and the absorption spectrum is as shown in line 2. To another cuvette containing 25 μmoles of potassium phosphate buffer of pH 6 in a volume of 1.35 ml, 0.15 ml of the total peroxide was added and an absorption spectrum of this solution is also shown in line 3. Line 4 was obtained by the addition of the 0.15 ml of the total peroxide, which was previously incubated with 0.05 μg of commercial catalase for 10 min at room temperature and is comparable to line 2.

ide may be involved in a phytopathological function (3, 10) protecting against microbial infections. But until more is known of its origin or of the synthetic system producing peroxide, the importance and function of the high concentration of the peroxide in poplar twigs cannot be assessed.

We suggest that the peroxide level in the tissue is dependent upon the growth phase of poplar, thus indicating that some synthetic reactions of peroxide may be functionally related to cellular metabolisms.

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