Disulfides in Bean Leaves Exposed to Ozone

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ABSTRACT

Primary leaves of pinto bean contained disulfides only after exposure to ozone. Disulfide formation was not accompanied by a corresponding decrease in sulfhydryl content. The normal, dark-induced decrease in sulfhydryl content may be inhibited by ozone. Phytopathology 60:1842-1843.

Additional key words: Polyvinylpyrrolidone, sodium sulfite.

Sulfhydryl groups, important to many vital processes, are destroyed when plants are damaged by high levels (100 pphm) of ozone (6). These sulfhydryl groups are probably oxidized to disulfides, but until now this has not been demonstrated. To demonstrate this, the sulfhydryl groups changed by ozone had to be differentiated from those changed in the normal metabolism of the plant. Normally, the sulfhydryl content of bean leaves increases in the light and decreases in the dark (1). Disulfides could also be produced by this process. To resolve this problem, we studied the effect of ozone on the concn of disulfides as well as sulfhydryl groups in bean leaves. The ozone concn (25 pphm) used in these experiments approximate an average of that found outdoors in the USA on days when air pollutants accumulate.

Pinto bean plants (*Phaseolus vulgaris* L.) 10-14 days old, with two primary leaves of similar size were selected. These plants were placed in a 75-liter fumigation chamber under 4,000 ft-c of light for 3 hr to open stomata and raise the sulfhydryl content to its max (1). One leaf was removed from each plant for control tissue. The opposite leaf was either exposed to 25 pphm ozone for 3 hr or treated as indicated. In each case, the analysis of the fresh tissue of one leaf was compared to that of the opposite leaf. Samples were ground in an ice-cooled mortar containing a small amount of clean, white sand and 2 ml of cold phosphate buffer (1) alone or with either PVP (polyvinylpyrrolidone, Mann Laboratories, mol wt 10,000), or sodium sulfite 2.5% (w/v), or both. Because freezing may change sulfhydryl content by denaturing proteins (5), freezing was avoided during the preparation of bean leaf tissue for analysis. PVP was used to prevent the destruction of sulfhydryl groups by phenolic groups present in the bean leaves (2). Disulfides were detected by the addition of sodium sulfite which reduces disulfides to sulfhydryl groups. Therefore, disulfides can be measured as an increase in the sulfhydryl content of preparations treated with sodium sulfite (3). The sulfhydryl content was measured by titration with silver nitrate (1).

TABLE 1. The effect of 3 hr of ozone (25 pphm) and an 18-hr dark period on sulfhydryl content (μmoles/g fresh wt) of opposite bean leaves, A and B, ground in polyvinylpyrrolidone medium without sodium sulfite[a]

Leaf A	Leaf B	Mean difference
Light	*Light and Ozone*	
1.35	1.40	
1.40	1.45	
1.40	1.50	0.066[b]
Light	*Light, then dark*	
1.35	1.10	
1.50	1.20	
1.40	1.15	0.266[c]
Light and ozone	*Light and ozone, then dark*	
1.50	1.40	
1.50	1.30	
1.40	1.25	0.15[d]

[a] Light periods with and without ozone were 3 hr.
[b] Not significant.
[c] Significant beyond 1% level.
[d] Significant beyond 5% level.

Leaves were not visibly injured immediately after ozonation, but some injury developed 18 hr later.

When opposite leaves, neither of them ozonated, were ground in the same medium, the average difference in sulfhydryl content was 0.00 ± 0.05 μmole/g. The difference between samples of nonozonated, opposite leaves ground with PVP and samples ground with sodium sulfite was only 0.00 ± 0.05. After ozonation,

TABLE 2. The sulfhydryl content (μmoles/g fresh wt) of opposite bean leaves, A and B, before and after exposure to ozone (25 pphm for 3 hr)

Control[a]		Ozonated[a]		Ozonated, then dark[a]	
Leaf A	Leaf B	Leaf A	Leaf B	Leaf A[a]	Leaf B[a]
1.45	1.45	1.35	1.50	1.20	1.35
1.45	1.50	1.35	1.60	1.35	1.50
1.50	1.55	1.50	1.60	1.40	1.50
1.60	1.55	1.70	1.90	1.45	1.60
1.70	1.70	1.75	1.95	1.50	1.70
1.75	1.75	1.75	1.95	1.50	1.80
1.75	1.80	1.80	1.95	1.60	1.90
		1.85	2.00	1.65	1.85
Md = 0.014[b]		Md = 0.175[c]		Md = 0.1875[c]	

[a] Leaf A ground in polyvinylpyrrolidone medium without sodium sulfite. Leaf B ground in same medium with sodium sulfite. Control leaves not exposed to ozone or subjected to dark. Dark period is 18 hr following exposure to ozone. Md = mean difference.
[b] Not significant.
[c] Significant beyond 1% level.

the sulfhydryl content of samples of opposite leaves ground with sodium sulfite increased an average of 0.15 ± 0.00 μmole/g.

Table 1 shows the effect of ozone and a dark period on the sulfhydryl content of bean leaves. The sulfhydryl content of leaves not exposed to ozone was about the same as leaves immediately after ozonation. During the dark period, the sulfhydryl content of leaves not exposed to ozone decreased 19%, while that of ozonated leaves decreased only 10%.

Table 2 presents the measurement of sulfides as determined by the addition of sodium sulfite to the grinding medium. Disulfides were detected only in the ozonated leaves, and were present in these leaves even after 18 hr in the dark.

Evidence for disulfides in bean leaves was found only after they had been exposed to ozone. Ozone may either denature protein so that naturally occurring disulfides become accessible for sulfite cleavage, or it may produce disulfides by sulfhydryl oxidation. Since we found no concurrent decrease in sulfhydryl groups, such as that produced by high levels of ozone (6), our data indicate that low levels of ozone expose and oxidize sulfhydryl groups not detectable before ozonation. Our data also indicate that ozone may inhibit the decrease of sulfhydryl groups that normally occurs in bean leaves in the dark (1).

Sulfhydryl groups are involved in the permeability of membranes (4). The effect of ozone on sulfhydryl metabolism may be related to changes in membrane permeability produced by ozone (1).

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