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INDUCTION OF GLYCOLATE OXIDASE BY SO_2 IN *NICOTIANA TABACUM*

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Key Word Index—*Nicotiana tabacum*: Solanaceae; tobacco; glycolate; glycolate oxidase; SO_2 action; enzyme induction.

Abstract—In contrast to the inhibitory action of sulfite on glycolate oxidase, the specific activity of the enzyme in tobacco leaves exposed to SO_2 for 18 hr increases in proportion to the SO_2 concentration. This increase is strongly reduced by pretreatment with cycloheximide. As a consequence of induced *de novo* synthesis of glycolate oxidase the glycolate content of the leaves is markedly reduced after 18 hr exposure to SO_2.

INTRODUCTION

Sulfite, *in vitro* as well as *in vivo*, causes a marked inhibition of glycolate oxidase (EC 1.1.3.1) due to the formation of the hydroxysulfonate of glyoxylate (glyoxylate bisulfite) [1, 2]. This compound could be traced by fumigation of rice leaves with 10 ppm $^{35}SO_2$ [3]. The inhibition of glycolate oxidase results in an increase in the glycolate level when the leaves are either placed in 50–200 mM sodium bisulfite [4] or are supplied with 5 ppm SO_2 [5] whilst they fix CO_2. However, long-term fumigation (>10 hr) may increase the enzyme activity within the plant, either by reducing the rate of turnover or by changing the catalytic constants. These long-term increases in activity are imposed upon the actual inhibition, and persist after discontinuation of the SO_2 exposure. The present work was undertaken to investigate these changes in glycolate oxidase.

RESULTS

The control glycolate oxidase reaction is linear with time (Fig. 1), irrespective of whether an unpurified leaf extract or one purified with a Sephadex column was used. The average rate of activity for the leaf extract was 1.5 μmol glycolate/mg protein/hr. In the presence of 0.5 mM sulfite, during the first 40 min, 66% inhibition was caused and this rose to 100% with 1 mM sulfite. The activity then recovers, presumably due to the oxidation of sulfite.

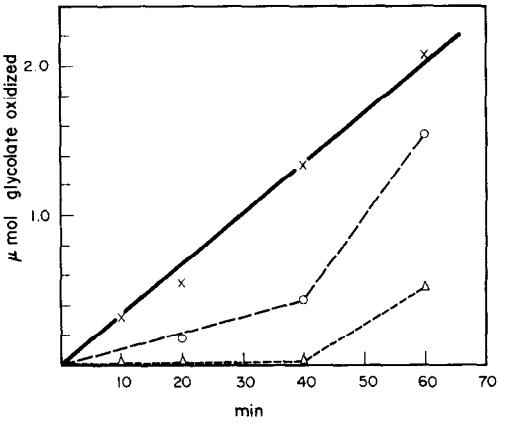

Fig. 1. Activity of glycolate oxidase in extracts of *Nicotiana tabacum*. ×——× control; ○– – –○ 0.05 mM sulfite; △- - - - -△ 0.10 mM sulfite.

In Table 1 the sp. act. from control and fumigated leaves are given. The data were obtained from independent experiments with different plants from which two leaves were taken at similar times for control and for fumigation, respectively. It is evident that fumigation with 1.3 ppm SO_2 for 18 hr caused an increase of 39%,

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Table 1. Specific activity of glycolate oxidase in leaves of *Nicotiana tabacum*

	Control	Fumigated plants	Control + cycloheximide pretreatment	Fumigated plants + cycloheximide pretreatment
	(μmol glycolate/mg protein/hr)			
Experiment 1	1.38	2.08	—	—
Experiment 2	1.75	2.22	—	—
Experiment 3	0.97	1.40	0.95	1.08
Experiment 4	1.25	1.72	1.50	1.65

Leaves were fumigated with 1.3 ppm for 18 hr.

regardless of the control leaves. Pretreatment with cycloheximide, which is known to inhibit the synthesis of inducible enzymes in the cytoplasm [6], drastically reduced the increase in sp. act. This indicates that the increase was not due to the formation of a more active enzyme species (e.g. by splitting of S—S bonds to SH groups) under the action of SO_2 but to enhanced enzyme synthesis. Fumigation of leaves for 18 hr at different SO_2 concentrations showed that the increase in sp. act. rose with the SO_2 concentration. At 0.2, 0.7 and 1.3 ppm SO_2, activities were respectively increasing by 3, 20 and 40% above the unexposed controls.

The amount of glycolate in control tissues averaged 1.15 μmol/g fr. wt (S.D. = ±0.25). Fumigation with 1.3 ppm SO_2 for 18 hr reduced the glycolate concentration from 1.15 μmol/g fr. wt to 0.72 μmol/g fr. wt (S.D. = ±0.15).

DISCUSSION

The inhibitory constants for the *in vitro* reaction agree with those given by Zelitch [1]. The reversal of inhibition within 60 min parallels the oxidation of sulfite. This demonstrates the equilibrium between glyoxylate and its SO_3^{2-}-addition complex (glyoxylate + SO_3^{2-} ⇌ glyoxylate-SO_3 complex). As the SO_3^{2-} concentration decreases (due to its oxidation), the concentration of the complex which is responsible for the inhibition also decreases. This interpretation is supported by the findings of Corbett and Wright [7], who demonstrated the chemical interaction of various aldehyde bisulfite addition compounds with the inhibitory glyoxylate bisulfite in the presence of enzymatically produced glyoxylate in the leaf. Thus, the increase in glycolate level during photosynthesis caused by the addition compound presupposes a permanent supply of SO_2 to maintain a steady level of SO_3^{2-}. The enrichment in glycolate is further enhanced by an increased formation inside the chloroplast [8].

However, prolonged fumigation (>10 hr) induces the synthesis of glycolate oxidase and this may at least partially counteract the SO_2 effect. After fumigation has ceased, it may even reverse it and result in sub-normal glycolate levels. The more, since increased glycolate formation inside the chloroplast also ceases as the SO_3^{2-} disappears.

Studies on the precultivation of larch at low SO_2 concentrations to adapt them to higher concentrations [9] indicate that adaptive mechanisms in the intact organism do exist. The induced synthesis of glycolate oxidase shown above points to adaptive mechanisms at the enzymatic level.

EXPERIMENTAL

Materials. *Nicotiana tabacum* was grown in the greenhouse. Two successive leaves from the mid-nodal region (*ca* 20 cm^2 size) were taken, cut again under H_2O and exposed to fumigation and to air, respectively, in small tubes covered with Parafilm.

Fumigation. For fumigation the Ecocal Calibration Unit Model 202 was used as the source of pollutant. The assembly generated 720 ng SO_2/min and the desired concn was achieved by dilution with carrier air (500 ml/min). The plants were exposed to SO_2 and to air, respectively, in glass containers of 1.2 l. content. The SO_2 concn was controlled by a Meloy FPD Sulfur Analyzer, Model SA 160-2. The containers were placed in a Heraeus incubator at 18° under continuous illumination with an Osram HQL 400 W lamp, 18 klx.

Treatment with cycloheximide. Before exposure to SO_2 and to control air, respectively, the stalks of cut leaves were immersed in cycloheximide (50 mg/l.) for 3 hr during illumination at 18 klx.

Assay of glycolate oxidase. The leaves were homogenized in a Potter-Elvehjem with Pi buffer (10 mM, pH 8.3) in a ratio of 1 g fr. wt/2 ml. The resulting extract was centrifuged for 10 min at 4000 *g* and the ppt. discarded. The extract (0.2 ml, corresponding to *ca* 2.5 mg protein) was incubated with the same Pi buffer and glycolate (3 mM final concn) in a total vol. of 3 ml and shaken at 30°. Aliquots (200 μl) were removed at intervals indicated and added to 50 μl 10 N H_2SO_4. After centrifugation, the decrease of glycolate was determined in 50 μl aliquots as described below. For purification the extract was passed through a column of Sephadex G 25 superfine.

Glycolate determination. Glycolic acid was extracted from leaves and purified by adsorption on Dowex 1 × 20 (200–400 mesh, acetate form) as described in [2], using the same size of column. The first 2 ml were discarded, the following 7 ml eluant (containing all of the glycolate) were pooled and glycolate was determined in 0.2 ml aliquots according to [10]. Impurities which may interfere with the colour reaction [11] were kept below the threshold concn.

Protein and chlorophyll determination. For protein determination by the naphthalene blue black procedure, 10–20 μl portions of the extract were spotted on squares (2 × 2 cm) of Whatman No. 1 paper and further treated as described in ref. [12]. Chlorophyll was determined according to ref. [13].

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