EFFECT OF SULPHUR DIOXIDE ON THE METABOLISM OF GLYCOLLIC ACID BY BARLEY (*HORDEUM VULGARE*) LEAVES*

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Abstract

Barley leaves treated with 5 p.p.m. SO_2 in the presence of $^{14}CO_2$ showed an accumulation of [^{14}C]glycollic acid and ^{14}C-labelled sugar phosphates together with a decrease in [^{14}C]sucrose and ^{14}C-labelled pigments compared with untreated leaves. It is proposed that the dissolution of SO_2 in the aqueous phase within the leaf leads to the formation of α-hydroxysulphonates which inhibit the enzyme, glycollate oxidase. The observed changes in ^{14}C distribution in sucrose, sugar phosphates, and glycollic acid can be related to such an inhibition.

Introduction

The effects of atmospheric SO_2 on plants have been investigated for many years and this work has been the subject of a number of reviews (Katz 1949; Thomas *et al.* 1950; Webster 1967; Guderian and van Haut (1970). Most of this work reports visual changes in leaf structure or quantitative changes in individual metabolites. However, the effects of SO_2 on the overall metabolic processes which go to make up both photosynthesis and respiration have been investigated; photosynthetic rate is retarded with increasing SO_2 concentration whilst respiration is increased or decreased depending upon the SO_2 concentration (Katz 1949; de Koning and Jegier 1968).

This paper discusses results of experiments in which the effect of SO_2 on the metabolism of $^{14}CO_2$ by barley leaves was investigated. $^{14}CO_2$ was used so that, by appropriate separation techniques, the individual ^{14}C-labelled metabolites could be determined and any changes in their relative concentrations due to SO_2 damage measured. This technique would thus enable the point(s) of attack by SO_2 in a particular metabolic sequence to be determined.

Materials and Methods

Seeds of *Hordeum vulgare* of unknown cultivar were obtained from commercial sources. Two seeds were germinated on cotton-wool soaked with a commercial full-nutrient solution. The seedlings were grown on a laboratory window ledge to the three-leaf stage prior to use. During this period they were watered with tap water. The plants were exposed to $^{14}CO_2$ in an apparatus

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shown diagrammatically in Figure 1. Moist air of a measured humidity was passed over two barley plants in each of two exposure chambers. To one of these chambers a small flow of 5 p.p.m. SO_2 in air could be added using the teflon permeation tube technique of Stevens *et al.* (1969). In a given experiment the plants were acclimatized to the exposure chambers for at least 1 hr prior to $^{14}CO_2$ addition. SO_2 was added to one of the chambers and this was followed by the addition of $^{14}CO_2$ in equal amounts to both chambers. SO_2 exposure times ranged from 15 to 60 min. The plants were grown for 30 min after the $^{14}CO_2$ pulse and then the leaves were rapidly removed from the plants and dropped into boiling 90% ethanol. The concentration of CO_2 in the experiment was that of the normal atmosphere supplemented by the addition of 8 p.p.m of CO_2 from the generation of $^{14}CO_2$. The experiments were carried out in sunlight in a laboratory with no additional artificial illumination. The relative humidity was maintained in the range 90–95% at room temperature in all experiments.

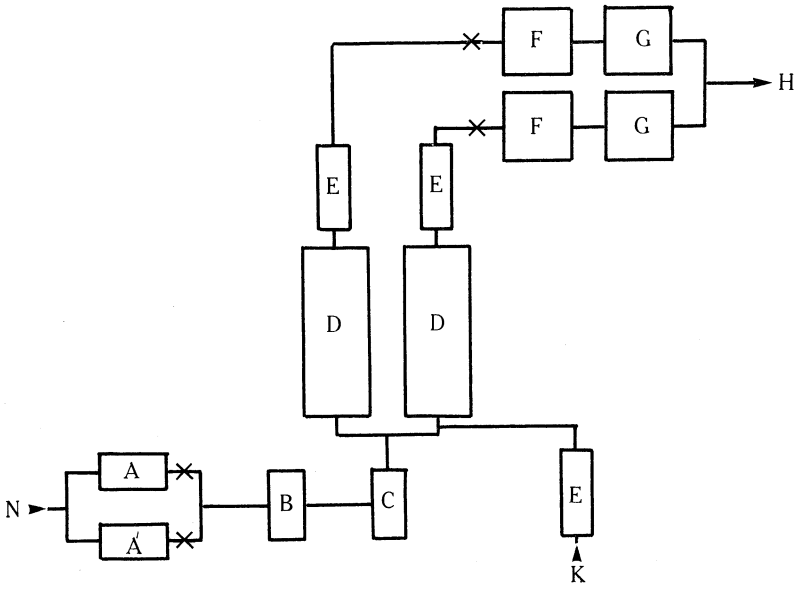

Fig. 1.—Block diagram of gas-flow apparatus used in the exposure of barley plants to $^{14}CO_2$ and SO_2. *A*, Dreschel bottle containing water; *A'*, Dreschel bottle containing silica gel; *B*, 10-ml three-necked conical flash containing 10% H_2SO_4; *C*, wet and dry bulb thermometer assembly; *D*, plant exposure chambers; *E*, rotameters; *F*, Dreschel bottles containing 1 vol. hydrogen peroxide; *G*, Dreschel bottles containing 0·1N NaOH; *H*, vacuum from water-pump; *K*, source of 5 p.p.m. SO_2 in air; *N*, compressed air; X, tap.

$^{14}CO_2$ was generated by adding 1 ml of $NaH^{14}CO_3$ solution (20 μCi; of specific activity 40 mCi/mmole) to 5 ml, of 10% (v/v) sulphuric acid in a 10-ml three-necked flask. The $^{14}CO_2$ was carried in equal air flows through both exposure chambers, the residue being collected in 0·1N sodium hydroxide.

The exposed leaves were extracted twice with 80% ethanol and twice with distilled water in a teflon and glass tissue homogeniser. Both ethanol and water extracts were reduced in volume under vacuum and chromatographed separately on Whatman No. 4 chromatography paper in phenol–water (100: 30 w/w) and butanol–propionic acid–water (375: 176: 250 v/v) solvents (Wilson and Calvin 1955). The developed chromatograms were autoradiographed against X-ray film for 1 week. Identification of ^{14}C-labelled compounds was by co-chromatography.

All ^{14}C-labelled compounds were cut from chromatograms in which good separation had occurred and were counted in a liquid scintillation spectrometer by placing the cut portions of the chromatogram in liquid scintillation counting vials containing NE 213 (Nuclear Enterprises Ltd). Quench corrections were applied using the channels-ratio method.

Results and Discussion: Changes in ^{14}C-labelling

In the absence of SO_2 the following ^{14}C-labelled products were found: sucrose, serine, sugar phosphates, malate, citrate, alanine, aspartate, glutamate, glycine, chlorophyll *a*, chlorophyll *b*, β-carotene, and phaeophytin. With 5 p.p.m. of SO_2 in the exposure chamber the appearance of [^{14}C]glycollate was also noted. Table 1 summarizes the average percentage distribution of ^{14}C amongst the compounds detected by autoradiography in both ethanol and water extracts. In this table compounds containing less than 1% of the total ^{14}C fixed are omitted and the pigments and sugar phosphates are considered as a group rather than as individual compounds. It can be seen that the mean percentage distribution of ^{14}C in any compound varies with the time of exposure to SO_2. The significance of the difference between the means was tested using Student's *t*-test and the results of this analysis are also presented in Table 1.

The data in Table 1 have been used to estimate the changes in ^{14}C distribution to be expected between SO_2-treated and untreated leaves at the 95% and 90% confidence levels. These expectations are shown in the following tabulation:

Confidence level	Extractant	Glycollate	Sugar phosphates	Sucrose	Pigments
95%	Ethanol	+12%			
95%	Water	+2%	+5%		
90%	Ethanol	+15%		−1%	−1%
90%	Water	+2%	+7%	−6%	

The changes in the ^{14}C distribution in glycollic acid must be regarded as minimum changes because glycollic acid is known to be volatile and thus subject to loss during chromatography, autoradiography, and storage. It can be readily seen that the greatest expected change is associated with glycollic acid.

A method frequently used in the study of glycollate metabolism has been to inhibit the enzyme glycollate oxidase. Zelitch (1957, 1958, 1959) and Zelitch and Walker (1964) have shown that α-hydroxysulphonates are extremely effective competitive inhibitors of glycollate oxidase activity. α-Hydroxysulphonates are bisulphite addition compounds of aldehydes and ketones and it was shown that the inhibition of glycollate oxidase activity by bisulphite was due to the formation of such an addition compound (sulphoglycollate) with glyoxylate. It would thus appear that the observed accumulation of [^{14}C]glycollic acid in barley leaves treated with $^{14}CO_2$ in the presence of 5 p.p.m. SO_2 was due to the formation of sulphoglycollate from glyoxylate and sulphur (IV) species formed when SO_2 dissolved in mesophyll water. Recently Tanaka *et al.* (1972) have found glyoxylate bisulphite in rice leaves exposed to SO_2 and have also suggested that this would inhibit glycollate oxidase. This mechanism can also be used to explain the decrease in ^{14}C-labelled sucrose in the presence of SO_2. The inhibition of photorespiration by the inhibition of glycollate oxidase would result in an inhibition of serine formation by this mechanism. Most

TABLE 1

AVERAGE PERCENTAGE DISTRIBUTION OF ^{14}C AMONGST COMPOUNDS EXTRACTED FROM BARLEY LEAVES EXPOSED TO $^{14}CO_2$ IN THE PRESENCE AND ABSENCE OF SO_2

Standard deviations given in parentheses

SO_2 concn. (p.p.m.)	Average percentage distribution of ^{14}C in									No. of experiments averaged
	Sucrose	Sugar phosphates	Malate	Glycollate	Aspartate	Glutamate	Alanine	Serine	Pigments	
					^{14}C-labelled compounds extracted with water					
0	67 (9)	12 (3)	3 (3)	0 (0)	11 (3)	4 (6)	3 (3)	1 (3)	0 (0)	6
5*	52 (4)	26 (9)	5 (3)	5 (3)	7 (5)	1 (1)	3 (2)	3 (4)	0 (0)	5
Difference†	−15	+14	−2	+5	−4	−3	0	+2	—	
t‡	3	3·5	1·4	3·5	1·4	1·0	—	0·9	—	
					^{14}C-labelled compounds extracted with ethanol					
0	56 (16)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	13 (12)	0 (0)	30 (10)	6
5*	34 (16)	0 (0)	0 (0)	29 (17)	0 (0)	0 (0)	20 (7)	0 (0)	16 (9)	4
Difference†	−22	—	—	+29	—	—	+7	—	−14	
t‡	2	—	—	3·8	—	—	1	—	2	

* Period of exposure to SO_2 = 30 min.

† An increase in percentage ^{14}C in a compound from the SO_2-treated leaf is regarded as positive, and a decrease as negative.

‡ Student's t-test on the difference.

of the serine produced in photorespiration is converted into carbohydrate (Goldsworthy 1970), especially sucrose (Tamas and Bidwell 1970). It is thus to be expected that sucrose formation would be inhibited if photorespiration was inhibited. Similarly, an accumulation of ^{14}C from $^{14}CO_2$ in sugar phosphates is to be expected if photorespiration is inhibited by glycollate oxidase inhibition as it appears likely that glycollic acid is derived from the sugar phosphates of the Calvin cycle (Goldsworthy 1970). The inhibition of photorespiration by SO_2 is unlikely to have any harmful effect on plants as photorespiration is a wasteful process involving the loss of CO_2 and energy (Goldsworthy 1970). Unless the accumulation of glycollate has some harmful effect, the action of SO_2 on glycollate metabolism is to the advantage of the plant.

Bisulphite and α-hydroxysulphonates are also known to have other effects on plant metabolism. Both α-hydroxysulphonates and the sulphite ion inhibit photosynthesis of isolated chloroplasts, probably by inhibiting photophosphorylation (Asada *et al.* 1965). However, it appears that 50% or greater inhibition of glycollate oxidase occurs at an inhibitor concentration of 10^{-4}M (Zelitch 1957) while a similar inhibition of chloroplast $^{14}CO_2$ fixation occurs at an inhibitor concentration of 10^{-3}M. It would thus be expected that glycollate oxidase activity would be affected by atmospheric SO_2 before photosynthetic CO_2 fixation. Tanner and Beevers (1965) found that α-hydroxysulphonates severely reduced respiration of *Ricinus communis* endosperm producing 50% inhibition of oxygen uptake at 10^{-3}M inhibitor concentration while no accumulation of glycollic acid occurred due to inhibition of glycollate oxidase activity. At a concentration of 10^{-3}M the α-hydroxysulphonates thus act as inhibitors of some step(s) of the respiratory processes of *R. communis* endosperm before affecting glycollate oxidase from this source.

Acknowledgments

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