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MINERALIZATION OF SULPHUR IN SULPHOQUINOVOSE BY FOREST SOILS

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Summary—Surface soils from four watersheds located in the Coweeta Basin near Franklin, North Carolina were assayed for their capacity to mineralize sulphur in 6-sulphoquinovose. All soils rapidly converted S in this component of the plant sulpholipid to inorganic sulphate, a soluble (salt extractable) ester sulphate and an insoluble ester sulphate. Sulphur in this latter fraction was released by acid-hydrolysis of soil residues at 121°C. Although maximum concentrations of S in each fraction varied with duration of incubation, rates of conversion of S into all fractions were highest during the first hour. Mineralization rates based upon sulphate release and total S released from sulphoquinovose are reported.

INTRODUCTION

Sulphoquinovose (6-sulpho-6-deoxyglucose) is the S-containing moiety of the plant sulpholipid (Daniel *et al.*, 1961) found in algae and green plants (Benson, 1963; Shibuya *et al.*, 1963) and in high concentration in photosynthetic tissues (Harwood, 1980) and to a lesser extent in non-photosynthetic tissues (Galliard, 1968; Stuiver *et al.*, 1978). Since the total S content of leaves is comprised primarily of sulpholipid and protein (Harwood and Nicholls, 1979), it is possible that significant quantities of this lipid (or its degradation products) enter soil during deciduous senescence. Indeed, based upon estimates of annual synthesis and turnover rate, Harwood and Nicholls (1979) consider the sulpholipid to represent an important source of soil sulphate. This possibility should be especially true for hardwood forests. Since available evidence (Harwood, 1980) indicates that plant tissues degrade the sulpholipid primarily to sulphoquinovose, it is likely that the latter serves as the primary substrate for S mineralization by the soil microflora. We report the mineralization by forest soils of sulphur in sulphoquinovose.

MATERIALS AND METHODS

Preparation of 6-sulphoquinovose

The ^{35}S-labelled plant sulpholipid was isolated from *Chlorella vulgaris* according to the procedure of Benson *et al.* (1959) except that chloroform-methanol (2:1 by vol) was employed as the final extractant (Lee and Benson, 1972). After deacylation in 0.3 M-methanolic KOH, the aqueous extract, containing glyceryl sulphoquinovose and three additional radioactive components, was treated with 4 N HCl for 2 h at 121°C to remove glyceryl residues and to hydrolyse any sulphate ester linkages present in the extract. The sulphonate linkage in sulphoquinovose is resistant to acid attack at elevated temperatures (Busby, 1966). As anticipated from the results of Benson *et al.* (1959), no apparent structural modification in the hexose moiety occurred since, after acid treatment and neutralization, apart from sulphate only one other radioactive component was detected by chromatography in a number of solvents (Benson *et al.*, 1959; Wolfersberger and Pieringer, 1974). This component had R_f values identical to those reported for 6-sulphoquinovose and had a mobility during electrophoresis identical to that for D-glucose 6-*O*-sulphate. The latter was prepared as described by Fitzgerald *et al.* (1971). Trace amounts of radioactive sulphate were removed from the extract as $BaSO_4$ by the addition of K_2SO_4 (as a carrier) and barium acetate. Excess Ba^{2+} were removed as $BaCO_3$. The preparation was not degraded by self-irradiation during our study.

Mineralization studies

Soil samples (0–5 cm, A-horizon) were taken from four watersheds at the Coweeta Hydrologic Laboratory, near Franklin, NC representing a mixed, mature hardwood forest (WS2); a 25.yr old white pine plantation (WS17) and two clearcut forests differing in duration of hardwood re-growth (WS7, 3 yr; WS48, 1 yr). A description of these sites and sampling have been given by Swank and Douglass (1977) and Fitzgerald *et al.* (1982), respectively.

Soils (1 g, wet wt) were incubated at 30°C with 7.5 nmol of ^{35}S-labelled 6-sulphoquinovose for varying periods up to 48 h. Mineralization of the ^{35}S-label was stopped by extracting the soil with 1 M Na_2SO_4 and salt-extractable ^{35}S (inorganic and ester sulphate) was completely recovered by further extraction of the sample with 1 M NaH_2PO_4 and 1 M LiCl. The soil residue was subsequently treated with 6 N HCl at 121°C for 12 h to hydrolyze any ester sulphate. Procedures for salt extraction and acid hydrolysis were described by Fitzgerald *et al.* (1982). Salt extracts were combined and analysed together with hydrolysates for total radioactivity using a Beckman LS9000 Scintillation counter. These fractions were also analysed for sulphate by electrophoresis of subsamples (10 μl) for 2 h at 250 V on Whatman No 1 paper in barium acetate–acetic acid buffer, pH 4.5. Under these conditions, radioactivity due to sulphate

Table 1. Mineralization of sulphur after incubation of soils from the Coweeta basin with 6-sulphoquinovose

Watershed	Incubation period (h)	^{35}S (nmol g^{-1} dry wt soil) converted to: SO_4^{2-}	Extractable ester SO_4^{2-}	Non-extractable S[a]	Total ^{35}S recovered after incubation (%)
2	1	1.15	0.73	0.66	90.3
	24	0.85	0.66	1.75	91.3
7	1	1.20	0.66	0.77	98.6
	24	0.98	0.71	1.54	100.2
17	1	1.02	0.68	0.85	103.0
	24	0.82	0.56	1.88	94.8
48	1	1.40	0.72	1.13	97.9
	24	0.79	0.47	1.90	96.7

[a]Sulphur remaining in soil after salt extraction and released after treatment of soil residues with 4 N HCl for 12 h at 121°C.

remained at the origin as $BaSO_4$ and was determined in the scintillation counter after sectioning of the origin (section 1 cm wide) and transfer to 5 ml of ScintiVerse (Fisher Scientific). Combined salt fractions and hydrolyzates were also analyzed separately for other ^{35}S-labelled components (including unreacted 6-sulphoquinovose) by electrophoresis as above but in sodium acetate–acetic acid buffer, pH 4.5. After location of each component by scanning of strips with a radiochromatogram scanner (Fitzgerald *et al.*, 1982), the radioactivity due to relevant sections (1 cm) was determined by counting in 5 ml of ScintiVerse.

RESULTS

Irrespective of duration of incubation, salt extraction of soils from all watersheds released ^{35}S-labelled sulphate, 6-sulphoquinovose and one additional component. The ^{35}S in the latter was completely converted to inorganic sulphate by treating extracts with 4 N HCl for 12 h at 121°C. Since sulphate esters are unstable in acid at elevated temperature (Fitzgerald, 1978), this result suggests that the unidentified component contains S in this linkage. Analysis of soil-hydrolysates revealed that approximately 85% of the ^{35}S was released as sulphate again suggesting that the bulk of this fraction was present in soil as ester sulphate.

The S of 6-sulphoquinovose was rapidly converted to inorganic sulphate, extractable ester sulphate and non-extractable (insoluble) ester sulphate (Table 1). Soils which were autoclaved before incubation with 6-sulphoquinovose failed to release ^{35}S from this substance. As expected, zero time controls demonstrated that extraction with Na_2SO_4 effectively inhibited mineralization and that incubation was required for the release of inorganic sulphate. Moreover, incorporation of ^{35}S into the ester sulphate fractions could not be detected in the zero time controls and was detectable only in trace amounts after 10 min incubation. These data suggest, in the case of the insoluble fraction, that ^{35}S was bound to this fraction in a covalent rather than ionic linkage after prolonged incubation. This view is supported by unpublished data showing that incorporation of ^{35}S into this fraction is temperature dependent and inhibited by azide, erythromycin and kanamycin.

Peak concentrations of inorganic sulphate were detected after 1 h of incubation with soils from all watersheds but maximum amounts of non-extractable ester sulphate were not attained until after 24 h of incubation. The time-course for the conversion of the sulphur of 6-sulphoquinovose into this latter fraction is shown in Fig. 1a. It should be noted that between 32 and 37% of the added S was found as non-extractable (insoluble) ester sulphate after 24 h with soils from all watersheds. Figure 1 also shows that 22–27% of the label was released as inorganic sulphate during the first hour and that quantities of this anion subsequently decreased (Fig. 1c). It may be significant that the lowest amount of inorganic sulphate in all soils, except the sample from Watershed 2, was present after 18 h and that

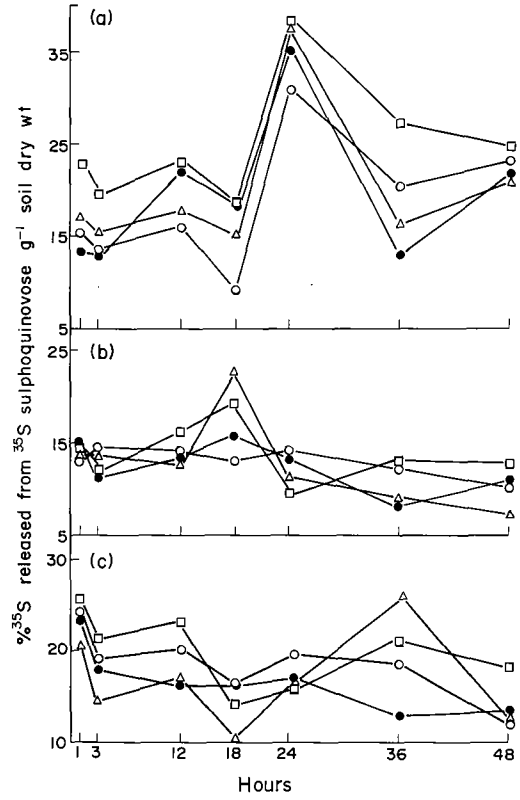

Fig. 1. The release and metabolic fate of sulphur in 6-sulphoquinovose incubated with soils from watersheds 2 (●), 7 (○), 17 (△), and 48 (□). (a) and (b), ^{35}S released and incorporated into non-salt extractable (insoluble) ester sulphate and extractable ester sulphate, respectively; (c), ^{35}S released as inorganic sulphate.

this loss coincided with peak amounts of extractable (soluble) ester sulphate (Fig. 1b). These latter concentrations subsequently declined during incorporation of the label into non-extractable ester sulphate. Although stoichiometry is lacking, the movement of the ^{35}S-label with respect to time (Fig. 1), suggests that inorganic sulphate is released before it is incorporated into extractable (soluble) ester sulphate which is then incorporated as such (or after hydrolytic release of sulphate) into the non-extractable (insoluble) fraction. The decline in this latter fraction in soils incubated with 6-sulphoquinovose for more than 24 h and the subsequent increase in inorganic sulphate concentrations suggests that insoluble ester sulphate is subject to turnover. The pattern for metabolic fate of the ^{35}S-label best illustrating these possibilities was observed with soils from watersheds 48 and 17 (Fig. 1).

Since inorganic sulphate must be released before ester sulphate is formed (Dodgson and Rose, 1970), it is felt that calculation of mineralization rates for S in 6-sulphoquinovose should take into account incorporation of released inorganic sulphate in addition to sulphate which remains un-incorporated. Although more sulphur was incorporated into the non-extractable fraction after 24 h for example, rates for release of inorganic sulphate and generation of ester sulphate (extractable and non-extractable) were fastest during 1 h incubations for soils from all watersheds (Table 1). Thus, rates during the first hour of incubation were 2.55, 2.63, 2.55 and 3.25 nmol ^{35}S released h^{-1} g^{-1} dry weight soil for watersheds 2, 7, 17 and 48, respectively.

DISCUSSION

In contrast to most naturally occurring organosulphur compounds where S predominates in an ester (C—O—SO_3^-) linkage (Dodgson *et al.*, 1982), sulphoquinovose is a sulphonate in which C is directly bonded to S as C—SO_3^- (Benson, 1963). To our knowledge, ours is the first report of the ability of soil to mineralize S in this form and the results suggest that this capacity may be widespread in forest soils irrespective of past management practices. Thus, soil samples from a hardwood forest converted to a monoculture-white pine stand 25 yr previously (Watershed 17) exhibited mineralization rates similar to those for undisturbed and clearcut hardwood ecosystems. In addition to generating inorganic sulphate, all watersheds rapidly incorporated S released from sulphoquinovose into salt-extractable (soluble) and non-extractable (insoluble) organic forms possessing sulphate ester linkages. These latter forms of S may result from the incorporation of released sulphate into organic matter via the sulphation of available hydroxyl groups by sulphotransferase enzymes of the soil microflora (Dodgson and Rose, 1970; Fitzgerald, 1978). Since the ^{35}S label was incorporated during the first hour of incubation, it is likely that pre-existing enzymes were involved to some extent. Although the liberation of sulphate alone is generally taken as an index of S mineralization, it is likely that a more realistic value will be obtained when the quantity of this anion as well as its metabolic products are taken into consideration. The use of a ^{35}S label has made this approach possible.

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