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SHORT COMMUNICATION

GENERATION OF SULPHATE FROM CYSTEINE IN FOREST SOIL AND LITTER

D. D. HALE and J. W. FITZGERALD*
Department of Microbiology, University of Georgia, Athens, GA 30602, U.S.A.

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Several transformations producing sulphate in soil have been studied, including sulphate ester hydrolysis (Fitzgerald and Strickland, 1987), S^0 and S^{2-} oxidation (Swaby and Vitolins-Maija, 1968) and oxidation of carbon-bonded S (Strickland and Fitzgerald, 1983; Fitzgerald and Andrew, 1984). Metabolism of this latter form of organic S, which includes amino acid S, may be of importance under certain circumstances. Thus, in soils lacking significant S^0 or S^{2-} oxidative capacity (Swaby and Fedel, 1973), mineralization of the S-containing amino acids may represent the major mechanism for the generation of sulphate and thus for the completion of the S cycle. In considering these amino acids as sources of soil sulphate, results of a seasonal study suggest that cysteine is more readily mineralized than methionine (Fitzgerald *et al.*, 1988). However, although the mechanism whereby cysteine S is converted to sulphate has been investigated (Freney, 1958, 1960), little information exists on factors governing this process. This is in direct contrast to information available on factors influencing transformations of methionine (Fitzgerald and Andrew, 1984, 1985). We have investigated cysteine transformations in forest floor layers and soil and have determined the effects of pH, substrate concentration and antimicrobial agents on the mineralization and immobilization of this amino acid.

For pH studies, soil was collected in February 1985 from the A1 horizon (0–5 cm) of each of 10 permanent plots established on watershed 18 at the Coweeta Hydrologic Laboratory, located near Otto, North Carolina. These equally spaced 0.01 ha circular plots are located at mid-elevation on a stream to ridge to stream transect. This catchment is a mixed mature hardwood forest with soil of the sandy loam Ashe series. For all other studies, samples were collected in June 1985 from the forest floor (01 and 02 layers) and the A1 horizon of plot 10. This plot is the one closest to the stream draining the catchment. Samples were stored (field moist) at 10°C in sealed polyethylene bags prior to analysis, following sieving to remove roots (< 1 cm, 02 and A1 horizon samples).

To determine mineralization and immobilization of amino acid S, samples (0.5 g 01, 02 and 1.0 g soil, wet weight) were exposed for 24 h at 30°C to L-cysteine (8 nmol S, unless otherwise indicated) as a mixture of the ^{35}S-labelled and unlabelled amino acid. Both processes were terminated simultaneously by isotope dilution after washing samples with 1 M Na_2SO_4 in a saturated solution of the amino acid followed by 2 washes with the saturated amino acid alone. Samples were then extracted 3 times each successively with 1 M NaH_2PO_4, 1 M LiCl, and water to recover adsorbed ^{35}S. The remaining ^{35}S was obtained by hydrolysis of the samples in 6 M HCl for 12 h at 121°C followed by extraction with 2 M NaOH. This salt, acid and base treatment recovered 85–95% of the added label. ^{35}S-labelled components in each extract were separated by electrophoresis, located on electrophoretograms by scanning and identified (Fitzgerald and Andrew, 1984). The radioactivity associated with each component, determined by liquid scintillation counting, was expressed as a percentage of the total radioactivity of the extract. The amount of each component, expressed as nmol S, was calculated from the amount of ^{35}S added initially. Activities were standardized to a 1 g dry weight basis. Mineralization potentials were determined from the amount of [^{35}S]sulphate present in all the extracts whereas potentials for incorporation of the amino acid into organic matter (immobilization) were determined from the amount of the ^{35}S-amino acid recovered in the acid and base extracts.

To determine the effect of pH on cysteine metabolism, approximately equal amounts (wet weight basis) of A1 horizon soil from each of the 10 plots were pooled and 1 g subsamples were added to sintered glass filter sticks. To each subsample an HCl solution (200 μl) of specified pH was added. Determinations were made in triplicate using deionized, distilled water (pH 5.6) and HCl solutions of pH ranging from 0.5 to 4.5. Samples were held for 48 h at 30°C to allow pH equilibration before an additional 400 μl of deionized, distilled water was added to simulate the addition of the amino acid solution and subsequent water wash. After 24 h at 30°C, final pH values of the samples were determined by extraction with water to yield a 2:1 water:soil ratio. Mean pH values of soil extracts ranged from 3.2 to 5.8. The pH values chosen for study were 3.2, 3.8, 4.8 and 5.8, obtained by using HCl solutions of pH 0.5, 1.0 and 1.5 and water of pH 5.6, respectively. To determine pH effects on cysteine metabolism, samples were prepared and treated as above except for the addition after the 48 h equilibration of 200 μl of a solution containing 8 nmol S as ^{35}S-labelled and unlabelled amino acid. This was followed by a 200 μl water wash. Samples were extracted after 24 h and assayed for mineralization and immobilization activity. Potentials for incorporation of sulphate into organic matter were also determined (Fitzgerald and Andrew, 1984).

Electrophoretograms of most extracts showed three radioactive components: sulphate, non-metabolized cysteine and an oxidation product of the amino acid. Sulphate detected in salt extracts was generated from cysteine while that recovered by acid and base extraction represented a portion of mineralized S which had become incorporated (immobilized) into organic matter. The organic S which is formed in forest soil after exposure to sulphate has been characterized in some detail (David and Mitchell, 1987). Mineralization activity reported here is the sum of values for sulphate found in salt as well as acid and base extracts. The presence of cysteine in these latter extracts indicated (Fitzgerald *et al.*, 1984) that some of the added amino acid was also incorporated into organic matter. This possibility for methionine was confirmed by direct exposure to and

*To whom all correspondence should be addressed.

Table 1. Effect of pH on cysteine transformation potentials in A1 horizon soil

pH	Mean (±SE, $n = 3$) transformation potential (nmol S g^{-1} dry wt)		
	Mineralization	Immobilization[a]	Incorporation[b]
5.8	12.6 (0.6)	0.6 (0.02)	1.7 (0.1)
4.8	10.1 (0.8)	0.6 (0.04)	1.9 (0.1)
3.8	5.1 (0.6)	0.4 (0.03)	1.3 (0.2)
3.2	7.9 (1.5)	0.5 (0.03)	1.4 (0.1)

[a]Incorporation of amino acid into organic matter.
[b]Incorporation into organic matter of sulphate generated from mineralization.

subsequent isolation of organic matter containing the amino acid in co-valent linkage (Fitzgerald and Watwood, 1988).

Decreases in soil pH generally resulted in lowered potentials for the mineralization and incorporation of cysteine (Table 1). The greatest decrease in mineralization (60%) was at pH 3.8. Incorporation (immobilization) potentials for this amino acid were not affected by pH to as great an extent. Between pH 5.8 and 3.8, cysteine incorporation activities decreased by only 33%. The greater effect of pH on mineralization as opposed to incorporation was not unexpected because the former process is the predominant transformation for cysteine in forest soil (Fitzgerald *et al.*, 1988). In some instances, lowered soil pH resulted in increased potentials for the mineralization and incorporation of cysteine (i.e. mineralization between pH 3.8 and 3.2) as well as increased potentials (i.e. between pH 5.8 and 4.8) for the incorporation of sulphate that was released during mineralization. These findings may reflect differences in the pH optima of the enzymes needed for these transformations.

Approximately linear increases in cysteine mineralization and incorporation potentials were observed for 02 and A1 horizon samples exposed to increasing cysteine concentrations up to 64 nmol S (Table 2). The capacity for mineralization always exceeded the capacity for incorporation in both horizons at any concentration. Linear regression analysis showed mineralization rates of 02 horizon samples to be 7-fold greater than incorporation rates while mineralization rates in A1 horizon samples were about 17-fold greater than incorporation rates. Similarly in 72 h time course experiments, cysteine mineralization rates in the 02 horizon were 11-fold greater than incorporation rates by regression analysis whereas mineralization activity in the A1 horizon was 36 times the incorporation activity. Collectively, these results further support findings from seasonal assays made at one substrate concentration (Fitzgerald *et al.*, 1988) that metabolism of cysteine proceeds mainly via mineralization. With respect to incorporation into organic matter of sulphate generated from the mineralization of increasing amounts of cysteine, linear increases occurred in samples from both the 02 and A1 horizon (Table 2). These data suggest that increases in cysteine inputs into the 02 horizon as a consequence of deciduous senescence would coincide with increases in sulphate followed by incorporation of up to 20% of this anion.

Table 2. Influence of cysteine concentration on transformation potentials for this amino acid in 02 and A1 horizon samples

Cysteine added (nmol S)	Transformation potential (nmol S g^{-1} dry wt)					
	Mineralization		Immobilization[a]		Incorporation	
	02	A1	02	A1	02	A1
1	1.7	0.6	0.4	0.1	0.3	0.1
8	37.2	8.4	5.9	0.7	6.4	1.1
16	62.2	8.8	9.6	0.6	12.8	1.2
32	178.9	33.6	22.7	2.0	33.0	4.3
64	474.6	67.6	63.5	4.0	93.6	9.2

[a]See Table 1.

Table 3. Effect of antimicrobial agents on cysteine metabolism in 01, 02 and A1 horizons[a]

Additions	Transformation potential (nmol S g^{-1} dry wt)					
	Mineralization			Immobilization		
	01	02	A1	01	02	A1
None	5.4	21.4	5.1	4.7	5.1	0.7
Sodium azide	3.6	4.3	2.9	1.9	3.0	0.3
Candicidin	9.2	28.1	6.9	4.4	4.8	0.8
Chloramphenicol	6.7	16.6	6.6	4.1	3.5	0.5
Tetracycline	7.2	15.3	7.7	4.5	2.3	0.6
Erythromycin	7.6	21.0	7.2	4.6	4.8	0.5
All antibiotics	5.4	12.8	4.0	3.7	2.0	0.3

[a]Samples treated with a saturated solution (200 μl) of azide or each antibiotic separately or in combination for 48 h at 30 C before exposure to cysteine for an additional 24 h.

Treatment with antimicrobial agents influenced the metabolism of cysteine in litter and soil (Table 3). Incorporation of cysteine into organic matter was inhibited by all antibiotics with the exception of A1 horizon samples treated with candicidin. The lack of a significant effect by candicidin, an antibiotic effective against fungi and algae (Lampen, 1969), was not unexpected because numbers of these microorganisms may be low. With 01 horizon samples, sodium azide inhibited incorporation of cysteine to the greatest extent (60%) but treatment with a combination of the antibiotics was less effective (21% inhibition). Samples from this horizon exposed to azide possessed decreased cysteine mineralization activity (by 33%) while treatment with the antibiotics individually resulted in increased mineralization of cysteine. Treatment of 02 horizon samples with a combination of the antibiotics resulted in the greatest inhibition of cysteine incorporation (61%) although tetracycline, sodium azide or chloramphenicol inhibited incorporation by 55, 41 and 31%, respectively. Gram-positive bacteria and fungi did not appear to be of importance in cysteine incorporation, which was inhibited only 6% by erythromycin or candicidin, respectively. Sodium azide inhibited cysteine mineralization to the greatest extent (80%). Cysteine mineralization was also inhibited by treatment with a combination of the antibiotics (by 40%) as well as by tetracycline (by 29%) and chloramphenicol (by 22%). The increase in cysteine mineralization promoted by candicidin may be due to reduced competition between the Gram-negative tetracycline- and chloramphenicol-sensitive bacteria mediating the transformation and fungi present in this horizon. Gram-positive and Gram-negative bacteria were involved in cysteine incorporation in the A1 horizon, as evidenced by the decreased activity of samples treated with chloramphenicol (29%), erythromycin (29%) or tetracycline (14%). Treatment with either sodium azide or a combination of the antibiotics inhibited this transformation to the greatest extent (by 57%). Such treatments also decreased cysteine mineralization in this horizon (43% inhibition by sodium azide and 22% by a combination of antibiotics).

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