Soil Biol. Biochem. Vol. 17, No. 6, pp. 881–883, 1985
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0038-0717/85 $3.00 + 0.00
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SHORT COMMUNICATION

METABOLISM OF METHIONINE IN FOREST FLOOR LAYERS AND SOIL: INFLUENCE OF STERILIZATION AND ANTIBIOTICS

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(*Accepted* 15 *April* 1985)

In a study of the metabolism of ^{35}S-labelled L-methionine by forest soil, incubation-time courses indicated that mineralization of amino acid S had ceased after 24 h and that only 35% of the added S was mineralized (Fitzgerald and Andrew, 1984). These results are in direct contrast with those obtained in a study of the mineralization of S in the plant sulpholipid in which 85% of the added ^{35}S was mineralized after 24 h by soil from similar forests (Strickland and Fitzgerald, 1983). Whereas most of the remaining sulpholipid could be recovered as unreacted starting material, depending upon the forest examined, between 23 and 51% of the added methionine could only be recovered from soil by acid and base extraction and thus under conditions which extract organic matter. Because these treatments hydrolyze peptide bonds, Fitzgerald *et al.* (1984) suggested that the added methionine was initially incorporated during incubation into organic matter via peptide bond formation. The methionine content of the acid-base extracts decreased substantially after 48 h and this decrease coincided with increased S mineralization. Thus, apart from converting methionine S to SO_4^{2-}, forest soil can also incorporate the amino acid into organic matter. Moreover, this organic matter fraction may be subject to turnover in these soils releasing methionine for subsequent mineralization. Our objective was to provide support for the possibility (Fitzgerald *et al.*, 1984) that the incorporation process regulates mineralization by governing the amounts of methionine made available for mineralization. To achieve this end, we have utilized antibiotics which selectively inhibit either the incorporation or mineralization of this amino acid.

Samples of 01, 02 forest floor layers and A1 horizon soil were collected in triplicate from a hardwood forest in North Carolina (Swank *et al.*, 1984). Each sample was assayed once and means ($n = 3$) of results obtained with each horizon are reported. Each sample was incubated (1 g wet wt) at 28°C for 48 h with 7.5 nmol L-methionine (mixture of unlabelled and ^{35}S-labelled amino acids; 4.4×10^{13} Bq mmol^{-1}). After incubation, samples were extracted successively with 2 ml vols 1 M Na_2SO_4 in a saturated solution of L-methionine (2 mmol, Na_2SO_4) and twice with saturated L-methionine alone (1.8 mmol unlabelled amino acid, total). The samples were then washed with three 200 μl vols each of 1 M NaH_2PO_4, 1 M LiCl and water. The experimental design for extraction and washing of samples was identical to that employed by Houghton and Rose (1976). All fractions were combined to yield a salt extract. This extraction quantitatively recovered both soluble and adsorbed ^{35}S. ^{35}S remaining in the samples was released by hydrolysis of the residue in 400 μl 6 M HCl for 12 h at 121°C followed by successive extraction with three 200 μl vols water and two 400 μl vols 2 M NaOH. The residue was washed with water as above and the respective washes were combined to yield an acid and a base extract. Based upon total recoveries of added ^{35}S which were in excess of 95% in all incubations, this salt-acid-base extraction procedure was considered suitable for assessing the fate of methionine in forest floor and soil samples. Small volumes of concentrated extractants were utilized so that extract samples could be subjected to electrophoresis without prior concentration. ^{35}S-labelled components in salt and acid-base extracts were separated by electrophoresis toward the anode in sodium acetate and quantitated (Fitzgerald and Andrew, 1984). All values reported represent means of triplicate determinations.

Electrophoresis of salt and acid-base extracts from all samples indicated the presence of up to four major ^{35}S-labelled components (electrophoretogram scans are not shown for the sake of brevity). The component with the greatest mobility was identified as SO_4^{2-} (Fitzgerald and Andrew, 1984). A separate ^{35}S-labelled component was detected in all extracts which, after elution, was identical to L-methionine on the basis of co-electrophoresis and co-chromatography on paper in solvents described by Kolousek *et al.* (1975). The presence of this component in salt extracts probably represents L-methionine which was not metabolized after 48 h; whereas, the presence of [^{35}S]methionine as such in the acid-base extracts suggests that some of the methionine added to the samples was incorporated into organic matter protein during the incubation. It is unlikely that any of the methionine recovered by acid-base treatment could represent methionine that was simply adsorbed to organic matter because the ratio of unlabelled-to-labelled methionine in the preliminary salt wash was about 3×10^5:1 assuming no metabolism of the labelled amino acid. A third radioactive component was detected in all extracts and was identical in electrophoretic mobility (relative to that of SO_4^{2-}) to the performate oxidation product of L-methionine (Moore, 1963). Acid hydrolysis (see below) of salt extracts resulted in the partial conversion of this metabolite to methionine. Methionine sulphoxide is preferentially reduced to methionine under these conditions (Savige and Fontana, 1977) suggesting an identity for this metabolite as the sulphoxide of methionine rather than methionine sulphone. Stock [^{35}S]methionine (New England Nuclear) was at least 95% pure by electrophoresis and chromatography. Oxidation to the sulphoxide or to other degradation products by self-irradiation (Kolousek *et al.*, 1975) was essentially eliminated by dilution of the preparation immediately after delivery to an aqueous solution containing 30 nmol ml^{-1}. Initial purity was maintained by freezing. A fourth major radioactive metabolite was detected only in salt extracts of samples incubated for 48 h. This metabolite was not detected in samples incubated for shorter ($\leqslant$24 h) periods. The component remained at the origin during electrophoresis and was converted to methionine after hydrolysis of salt extracts from all samples in 6 M

Table 1. Distribution of ^{35}S-labelled methionine and methionine-derived metabolites in salt extracts before and after acid hydrolysis

Salt extract from:	Components separated by electrophoresis (% total radioactivity)			
	Origin component[a]	Methionine	Oxidation product	SO_4^{2-}
01 before hydrolysis	20.1	10.7	5.8	5.3
after hydrolysis[b]	3.4	30.2	2.4	4.1
02 before hydrolysis	12.7	9.7	9.1	15.9
after hydrolysis	2.7	19.6	4.0	19.0
A1 before hydrolysis	10.0	8.0	5.5	32.1
after hydrolysis	1.7	20.0	2.1	31.9

[a]Component which remained at the origin after electrophoresis.
[b]Treatment with 6 M HCl for 3 h at 121°C.

HCl for 3 h at 121°C (Table 1). These results suggest that salt extraction was capable of recovering a portion of the methionine (possibly in the form of small peptides) which had been incorporated into protein during incubation.

The ability of samples from 01, 02 forest floor layers and soil to incorporate methionine into salt extractable and acid-base extractable peptides or protein was essentially abolished by autoclaving or by prior treatment with sodium azide (Table 2). Moreover, the appearance of the oxidation product of methionine in acid-base extracts from all three samples was similarly inhibited (Table 2) suggesting that all of the above processes are biologically mediated. In the case of the oxidation product, it could not be determined if some of the methionine was initially oxidized and then incorporated into protein or whether the occurrence of this metabolite in acid-base extracts resulted from oxidation of some of the methionine during release from protein (Freney *et al.*, 1972). Certainly salt extractable amounts of the oxidation product were not diminished in sterilized samples which suggests, as expected, that methionine oxidation occurs non-enzymically during incubation. Levels of this metabolite were in fact elevated, especially in samples treated with sodium azide, which was probably due to the fact that more non-metabolized methionine was made available for oxidation under these conditions.

In 01 layer samples, the potential for incorporation of methionine into salt extractable peptides and acid-base extractable protein was several-fold greater than the ability to mineralize methionine S (Table 2). Acid-base extractable levels of methionine decreased by about 55% in samples treated with candicidin and this resulted in about a 2.5-fold increase in the ability to mineralize methionine S. This increase was no doubt due to the greater amount of methionine made available in candicidin-treated samples. The data summarized in Table 2 show that the ability to incorporate methionine into acid-base extractable protein was mediated primarily by candicidin-sensitive fungi or algae (Lampen, 1969); whereas, the generation of SO_4^{2-} from methionine was due primarily to tetracycline- and chloramphenicol-sensitive bacteria. Additionally, the incorporation of methionine into salt-extractable peptides was mediated by a separate group of microorganisms (probably Gram-positive bacteria) because this process was inhibited by erythromycin (Table 2); an antibiotic which failed to exert an effect on the other two processes. This inhibition again resulted in an increase in the ability to mineralize methionine S and it would appear that in the 01 layer both incorporation processes regulate conversion to SO_4^{2-} by controlling available methionine. The abilities to incorporate methionine relative to that for mineralization decreased in the 02 layer and in the soil, but unlike the 01 layer, the former processes were inhibited by the antibacterial antibiotics and not by candicidin (Table 2). In contrast, the conversion of methionine S to SO_4^{2-} in the 02 layer and soil was inhibited by most of the antibiotics tested, including candicidin. Collectively, these data suggest that the ability of a given antibiotic to inhibit the metabolism of methionine is not only process dependent but also depends upon the type of forest sample examined. For example, S-mineralization in the 01 layer was mediated by tetracycline- and chloramphenicol-sensitive bacteria. Because this process was insensitive to erythromycin (an antibiotic with specificity for Gram-positive bacteria), bacteria mediating S-mineralization in this horizon are probably Gram-negative. Lack of inhibition by candicidin suggests that fungi are not involved. S-mineralization in the underlying 02 horizon and soil was substantially inhibited by all antibiotics except chloramphenicol suggesting the involvement of bacteria and fungi during August, the month during which samples were collected. Seasonal variation in this pattern of microbial involvement might be anticipated but was not investigated.

In summary, it appears that the utility of methionine as a source of SO_4^{2-} for forest soil will depend upon the

Table 2. Influence of sterilization and antibiotics on the metabolism of exogenous L-methionine in the 01 and 02 forest floor layers and A1 horizon soil from a hardwood forest

Treatment Additions[a]	Salt extractable SO_4^{2-}[b] (nmol S g^{-1} dry wt)			Acid extractable oxidation product[c] (nmol S g^{-1} dry wt)			Methionine incorporated into protein (nmol S g^{-1} dry wt)					
							Salt extractable[b]			Acid extractable[c]		
	01	02	A1	01	02	A1	01	02	A1	01	02	A1
None	1.84	13.22	4.37	0.87	1.38	0.65	7.34	3.67	0.92	22.82	23.46	2.39
Autoclaving[d]	0.28	0.19	0.06	0.28	0.23	0.15	1.13	0.59	0.30	1.25	4.54	0.93
Sodium azide	0.57	0.45	0.19	0.12	0.34	0.13	0.40	0.38	0.07	1.61	2.96	0.87
Candicin	4.67	6.02	0.33	0.88	1.04	0.09	9.70	6.57	4.12	10.34	21.63	2.97
Tetracycline	0.72	2.77	0.45	1.02	0.53	0.29	0.72	1.11	0.15	19.36	10.30	1.22
Chloramphenicol	0.82	11.60	2.47	1.76	1.90	0.52	5.02	2.40	0.11	28.83	17.50	1.30
Erythromycin	6.63	6.59	3.94	1.07	0.52	0.45	1.69	1.05	0.14	25.96	6.88	0.81

[a]2 mmol sodium azide and 0.1 mmol each antibiotic added with methionine before incubation for 48 h at 28°C.
[b]Recovered after incubation by addition of 1 M Na_2SO_4 in a saturated solution of L-methionine followed by successive washes 3 times each with saturated methionine, 1 M NaH_2PO_4, 1 M LiCl and water.
[c]Recovered from salt extracted samples by hydrolysis in 6 M HCl (121°C for 12 h) followed by extraction twice with 2 M NaOH.
[d]121°C for 3 h with cooling before addition of methionine.

potential that microorganisms in the overlying forest floor layers have for re-incorporating methionine, released after deciduous senescence, back into protein. Data presented here reveal that this incorporation process is microbially mediated and the results complement those obtained by Freney *et al.* (1972) who found that the S-containing amino acids comprised a substantial proportion of soil organic matter.

Acknowledgements—This work was supported by the National Science Foundation. We are grateful to W. T. Swank of the Coweeta Hydrologic Laboratory (U.S. Forest Service) Otto, NC for making watershed 18 available for sampling. T. L. A. was supported by a University Wide Fellowship awarded by the Graduate School of the University of Georgia.

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