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DIVISION III — SOIL MICROBIOLOGY

Behavior of Free Amino Acids in Soil[1]

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ABSTRACT

The behavior of a mixture of amino acids in a soil environment was studied. Extractions were made with 80% ethanol. The extract was concentrated and then was analyzed for amino acids by gradient elution chromatography. After 1 hour of soil contact in the cold, at least some of each amino acid could be recovered, but the extraction was not efficient. Replicate soil flasks to which amino acids had been added were incubated at 28° C. under conditions that allowed for both CO_2 and amino acid analysis of the same flask. After 24 hours, substantial degradation had occurred but at least trace amounts of each of the added amino acids except threonine could still be detected. Beta alanine appeared on the 24-hour chromatogram although it was not among the amino acids added initially. Results of both chromatographic analysis and CO_2 collection suggested that nearly all of the added amino acids were degraded by the end of 96 hours. Separate studies using microbiological assay failed to confirm the persistence of threonine in soil as reported in the literature. The possibility that the beta alanine found in the soil environment was formed from aspartic acid decarboxylation was explored, but large additions of aspartic acid to soil did not result in substantial increases in beta alanine.

RECENTLY Putnam and Schmidt (5) reported on the occurrence of small but detectable quantities of free amino acids in soil. Soil amended with glucose and inorganic nitrogen yielded more amino acids and a greater variety of amino acids than control soil. The amount and nature of the free amino fraction obtained was considered to be a function not only of the interactions between microbial synthesis and degradation, but also the extent to which amino acids adsorbed to the soil could be extracted. The chromatographic techniques used in the work cited were applied in the present study to follow simultaneously the behavior of numerous amino acids in a soil environment.

EXPERIMENTAL PROCEDURES

Ten-milligram quantities of each of 12 amino acids were placed in solution and added to 100 g. of Waukegan loam (pH 6.4) obtained from the University Farm. The following amino acids were included: aspartic acid, glutamic acid, threonine, proline, alanine, valine, methionine, isoleucine, leucine, phenylalanine, glycine, and serine. After 1 hour incubation at 4° C., the soil was extracted with 80% ethanol, the extract was concentrated, and 1 ml. of the concentrate was analyzed for amino acids by gradient elution chromatography on Dowex 50-X4 resin. The procedures for extraction, concentration, and analysis were as described previously (5).

The persistence of amino acids exposed to normal microbiological activity in soil was investigated with a second mixture of amino acids at generally higher concentration. Glycine and serine were omitted to avoid overlapping peaks. Aspartic acid, glutamic acid, and threonine were each applied at concentrations of 30 mg. per 100 g. of soil; proline, alanine, valine, and phenylalanine at 20 mg. each; and methionine, isoleucine, and leucine at 15 mg. each. Replicate flasks were incubated at 28° C. while attached to a gas exchange train essentially as described by Waksman and Starkey (7) to allow for the collection of CO_2 evolved from the degradation of the added amino acids. After 24 hours, and again after 72 hours, duplicate flasks were detached and the contents were pooled for extraction and analysis of residual amino acids.

Microbiological assay of beta alanine was performed with *Saccharomyces intermedius* (ATCC 2360) after the procedure of Billen and Lichstein (2). Since this organism responds to pantothenic acid, an additional assay was made with *Lactobacillus arabinosis* 17/5 (1) to account for the amount of pantothenate present. Threonine was determined microbiologically with the technique of Schmidt and Starkey (6) using *Streptococcus faecalis* R and the medium of Mayernick and Ewald (4).

RESULTS AND DISCUSSION

The extract obtained with 80% ethanol after a mixture of amino acids had been in contact with soil in the cold for 1 hour yielded enough of each amino acid so that a series of well-defined peaks appeared on the chromatogram. The recovery data included in table 1 make it clear that the extraction was notably inefficient. Best recovery was noted for methionine and this was only about 50%; the more acidic amino acids were extracted much less efficiently. It should be mentioned that higher alcohol/soil ratios had been used in preliminary experiments and had not resulted in appreciably greater recovery of added amino acids. Adsorption effects, analogous to those noted for lysine and arginine on bentonite (5), probably are significant for all amino compounds in soil systems, and probably account for the difficulties encountered in extraction.

Subsequent analyses were made after exposure to microbiological activity, hence these results are a function of both microbial degradation and soil adsorption. At least trace amounts of each of the amino acids except threonine that had been added were still detectable after 24 hours, but recoveries were sharply reduced (table 1) as a result of the action of soil organisms. The recovery data indicate that all of the amino acids, with the possible exception of aspartic acid, were decomposed substantially during the first 24 hours of incubation. Aspartic acid was recovered in slightly greater percentage than after 1 hour, but this may be accounted for in the three-fold increase in the amount of aspartic acid added to the incubation series.

Table 1—Percent recovery from soil of amino acids after various incubation periods.

Amino acid	1 hour	24 hours	72 hours
Aspartic acid	1.4	5.5	0.2
Threonine	4.0	0.0	-
Proline	35.3	trace	trace
Glutamic acid	13.6	trace	-
Alanine	29.1	1.3	-
Valine	41.4	9.6	*
Methionine	50.7	0.3	-
Isoleucine	22.1	20.9	-
Leucine	44.1	6.9	-
Phenylalanine	32.7	26.4	0.1
Glycine	14.6	not added	not added
Serine	20.6	not added	not added

* Sample lost, small but measurable amount present.

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After 72 hours only very small amounts of aspartic acid, proline, valine, and phenylalanine were present in the extract (table 1).

Measurements of CO_2 evolved during incubation emphasize that the amino acids were dissimilated rapidly despite adsorption to the soil. CO_2 released from treated soils in excess of control soil is plotted in figure 1. Microbial activity was greatest during the first 48 hours, and most of the amino acids were degraded in that period. CO_2 evolution slowed in the period from 48 to 72 hours and then increased between 72 and 96 hours with but slight excess over the controls thereafter. Both CO_2 evolution and chromatographic analyses point to the likelihood that nearly all of the added amino acids were degraded by the end of 96 hours. This interpretation is consistent with the results of Greenwood and Lees (3), who reported that most amino acids were deaminated in 24 to 36 hours in a soil percolation system, and with the results of Wheeler and Yemm (8), whose data show essentially complete deamination of glycine and glutamic acid from soil percolates in 3 to 4 days.

The secondary peak of CO_2 release noted in figure 1 during the fourth day of incubation may have been due to the metabolism of carbon residues derived from certain of the deaminated amino acids or from delayed decomposition of possibly more resistant amino acids. If resistant amino acids were involved, these did not appear in the 72-hour extraction and must have been adsorbed so strongly as to avoid alcohol extraction. Essentially all added amino acid carbon, however, can be accounted for in terms of that evolved in 96 hours plus that assimilated by a soil microflora of the usual 20 to 30% efficiency (figure 1).

Greenwood and Lees (3) reported that threonine and methionine departed from the usual pattern of rapid decomposition of amino acids in soil. Using paper chromatography, those workers found threonine still present in appreciable quantities after 8 days' exposure to soil, and methionine was presumably of the same order of persistence. Our results do not confirm the resistance of methionine, for as seen in table 1 at least half of the added methionine could be extracted initially, but only a trace remained after but 24 hours. As threonine proved very difficult to extract in our experiments, it is conceivable that the bulk of that added may have remained intact during soil incubation even though this seems unlikely from the CO_2-evolution data. Further information on the persistence of threonine was provided by an additional experiment in which the degradation of 50 mg. of l-threonine in 100 g. of soil was followed by microbiological assay procedures. The results of the threonine assays presented in table 2 clearly demonstrate that threonine persisted only in sterile soil, and that its disappearance from soil of normal microbiological activity was very rapid. Perhaps the smaller soil samples and percolation systems used by Greenwood and Lees account for the persistence they reported, but all of our data support the view that both threonine and methionine are no more resistant to degradation in soil than any of the other amino acids studied.

Table 2—Persistence of threonine in sterile and nonsterile Waukegan l. as determined by direct microbiological assay of the soil.

Soil system	Incubation period	Milligrams l-threonine	
		Added initially	Recovered
Sterile	0 hrs.	50.0	53.0
Nonsterile	40 hrs.	50.0	3.1
Nonsterile	48 hrs.	50.0	1.6
Nonsterile	78 hrs.	50.0	0.5
Nonsterile	100 hrs.	50.0	< 0.1
Sterile	100 hrs.	50.0	48.0

Table 3—Beta-alanine formed during degradation of aspartic acid in soil after 24 hours.

Waukegan loam 50 g.	Aspartic acid mg./g.	Beta-alanine μg./g.	Pantothenic acid μg./g.
Trial 1	6	2.5	0.10
Trial 2	6	14.4	0.04

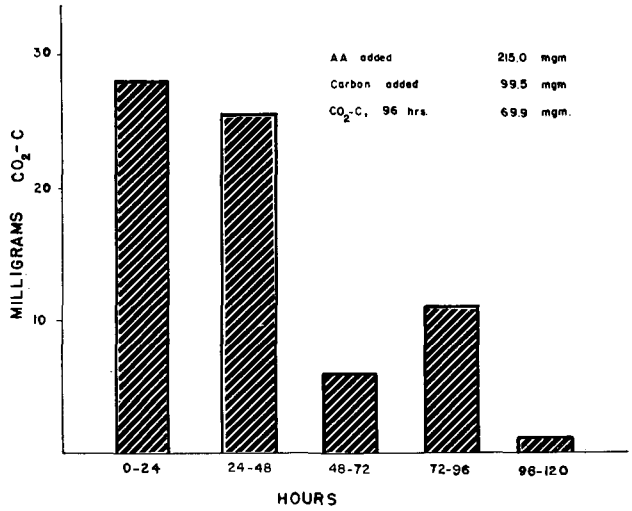

Figure 1—Carbon evolved as CO_2 during the incubation of soil to which 10 amino acids had been added. Values plotted are corrected for CO_2 evolved from control soil.

The chromatogram obtained for the 24-hour preparation had a peak at the beta-alanine position amounting to 2.6 μg. per g. of soil. As it was not among the 10 amino acids added to the soil and did not appear in preparations from unamended soil, it seemed likely that the beta-alanine was derived from the added amino acids. The fact that beta alanine is a moiety of the B-vitamin, pantothenic acid, lent additional interest to this observation. Certain bacteria are known to catalyze the decarboxylation of aspartic acid at the alpha carboxyl group to yield beta alanine (2). Microbiological assays were performed on soil following rather large additions of aspartic acid to confirm the occurrence of beta alanine and to test its association with aspartic acid degradation. The concentration of beta alanine after 24 hours is listed in table 3 together with that of pantothenic acid. Addition of large amounts of aspartic acid to soil did not influence the formation of beta alanine to any material degree, and the results gave no evidence of enrichment with organisms capable of alpha decarboxylation of aspartic acid. It is possible that the beta alanine found did result from aspartic acid decarboxylation, but that competition for substrate by other dissimilatory enzyme systems including beta decarboxylation, deamination, and transamination as noted by Billen and Lichstein (2) may have limited the yield. It is possible also that the beta alanine did not result from direct dissimilation of aspartic acid but rather that it was one of a number of excretion products completely resynthesized by microorganisms which used the aspartic acid merely as a nonspecific, readily assimilated substrate.

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