THE OXIDATION OF CYSTEINE TO SULPHATE IN SOIL

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Summary

The oxidation of cysteine to sulphate by a mixed population of soil micro-organisms was studied in a perfusion unit. Intermediates in this reaction were identified by paper chromatography and by an enrichment–reperfusion technique.

The results obtained by these two methods suggest that cysteinesulphinic acid, cysteic acid, sulphite, and β-hydroxypyruvic acid were involved in this oxidation. Sulphide, which has often been postulated as an intermediate in the conversion of organic sulphur to sulphate, could not be detected in the system nor in the gases emanating from the system.

I. Introduction

Much of the work performed on the decomposition of organic sulphur compounds which may occur in soil has been effected with pure cultures of micro-organisms (see Frederick, Starkey, and Segal 1957). These species may be unimportant members of the vast community of soil organisms. Also it is possible that the kinetics and pathway of sulphophication in soil are completely different from those relating to pure cultures. While there can be no question of the great importance of studies with pure cultures, it is obvious that if we wish to know which reactions are important in soil then it is necessary to perform the study with the soil itself.

For these reasons an investigation was conducted into the oxidation of cysteine to sulphate in soil contained in a perfusion unit. An earlier report (Freney 1958) showed that cystine and cystine disulphoxide were involved in this transformation. This paper reports the results of experiments designed to identify further intermediates in this reaction.

II. Experimental

The transformation of cysteine to sulphate in soil was studied in a perfusion unit of the type described by Lees and Quastel (1944). The principle of this technique has been adequately described by these two authors (1944, 1946).

In the system under investigation, 400 ml of 0·01M cysteine hydrochloride solution was perfused through 30 g of air-dried soil crumbs (2–5 mm fraction). The solution was cycled at such a rate that waterlogging of the soil did not take place. The soil used in most of these studies was a reddish brown clay loam derived from basalt.

Amino acids in the perfusate were detected, and a preliminary identification made, by two-dimensional paper chromatography. Details of the methods used were recorded in a previous paper (Freney, Delwiche, and Johnson 1959). In

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addition, amino acids containing sulphur were detected by an iodine–azide reagent (Block, Durrum, and Zweig 1958). Larger amounts of amino acids were isolated by chromatography on Whatman No. 3 paper (Block, Durrum, and Zweig 1958) in *n*-butanol–acetic acid–water (4 : 1 : 5 v/v) and *tert.*-butanol–water (7 : 3 v/v).

Keto acids in the perfusate were isolated as the 2,4-dinitrophenylhydrazones and identified by conversion to their respective amino acids by catalytic hydrogenation (Towers, Thompson, and Steward 1954; Meister and Abendschein 1956).

TABLE 1

R_F VALUES FOR CYSTEINESULPHINIC AND CYSTEIC ACIDS IN A NUMBER OF CHROMATOGRAPHIC SYSTEMS

Amino Acid	Whatman Paper No.	Solvent (v/v)	R_F
Cysteinesulphinic acid	4	*n*-Butanol–acetic acid–water (4 : 1 : 5)	0·16
	4	*tert.*-Butanol–water (7 : 3)	0·33
	1	Phenol–water (4 : 1)	0·13
	3	*n*-Butanol–acetic acid–water (4 : 1 : 5)	0·11
Cysteic acid	4	*n*-Butanol–acetic acid–water (4 : 1 : 5)	0·10
	1	Lutidine–collidine–water (1 : 1 : 1)	0·46
	1	Methanol–water–pyridine (20 : 5 : 1)	0·44
	1	*n*-Butanol–pyridine–water (1 : 1 : 1)	0·26

The enrichment–reperfusion technique (Gleen and Quastel 1953) was used to provide further evidence that certain compounds were intermediates in this transformation. Briefly, this technique involves perfusing a solution of cysteine hydrochloride through soil until sulphate production reaches a maximum. Sulphate is then removed from the perfusion system by washing with water. As a result of this treatment the soil has a population of microorganisms and a system of enzymes capable of immediately oxidizing to sulphate, cysteine, or any of the intermediates in its transformation to sulphate, i.e. without a lag phase. (Soil thus treated will be referred to as the enriched soil.) If the substance reperfused after cysteine is not an intermediate in this transformation then a lag phase would occur while systems are built up which are capable of oxidizing the new substrate to sulphate.

Sulphate was determined by Johnson and Nishita's reduction–methylene blue method (1952). The soil in the perfusion unit was analysed for sulphide by digesting the soil with aluminium metal and hydrochloric acid. Any hydrogen sulphide evolved was determined by the methylene blue method. The gases emanating from the perfusion system were passed through a trap containing zinc acetate, and the contents of the trap were analysed for sulphide plus sulphate by Johnson and Nishita's method. The zinc nitroprusside reaction described by Feigl (1947) was used to detect sulphite.

The results obtained with the soil described above were confirmed with other soils. The soils used in this study were:

(1) Dublin soil, California, U.S.A.
(2) Reddish chocolate soil derived from basalt, Walcha, N.S.W.
(3) Lateritic podzolic soil derived from orstein and granite, Uralla, N.S.W.
(4) Krasnozem derived from basalt, Lismore, N.S.W.
(5) Red-brown earth derived from sediments, Griffith, N.S.W.
(6) Solodic soil derived from sediments, Ashford, N.S.W.
(7) Lateritic podzolic soil derived from granite, Beerwah, Qld.
(8) Chocolate soil derived from basalt, Merriwa, N.S.W.

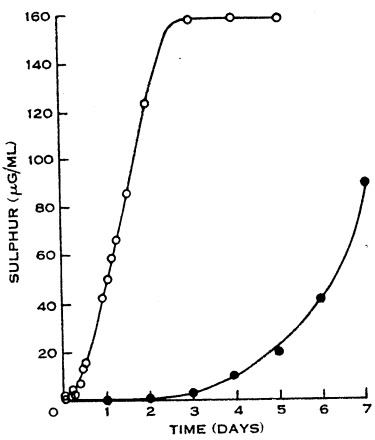


Fig. 1

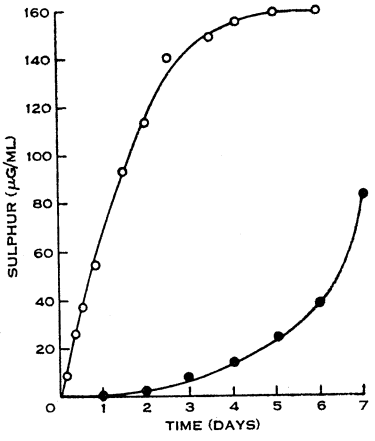


Fig. 2

Fig. 1.—Sulphate production from a 0·01N cysteinesulphinic acid solution perfused through an enriched soil (○) and a normal soil (●).

Fig. 2.—Sulphate production from a 0·01N cysteic acid solution (pH adjusted to 7) perfused through an enriched soil (○) and a normal soil (●).

The results obtained in the perfusion unit were compared with those obtained in a soil *in situ*. A cysteine hydrochloride solution was applied to a soil in the field, and the area covered with a plastic sheet to prevent leaching by rain. After a period of 48 hr, the area was sampled and the samples extracted with normal hydrochloric acid. Amino acids in the evaporated extract were detected by paper chromatography.

The sulphur-containing amino acids were obtained from the California Foundation for Biochemical Research, Los Angeles, U.S.A., and other amino acids from British Drug Houses Pty. Ltd., Poole, England.

III. Results

Chromatograms of the perfusate revealed the presence of a number of amino acids. One of these amino acids and cysteinesulphinic acid ran as a single spot when chromatographed together under a variety of conditions. Another amino acid appeared to be cysteic acid when tested by a similar procedure. The R_F values found for these amino acids under these conditions are given in Table 1.

When 0·01N solutions of cysteinesulphinic acid and cysteic acid (adjusted to pH 7 with sodium hydroxide) were reperfused through enriched soils, sulphate was produced immediately. However, when these solutions were perfused through untreated soil, sulphate was produced only after a lag period of 2 days (see Figs. 1 and 2).

In addition to the two amino acids containing sulphur, three other amino acids (serine, alanine, and glutamic acid) were identified in the perfusate by co-chromatography. The corresponding keto acids (β-hydroxypyruvic acid, pyruvic acid, and α-ketoglutaric acid) were detected by chromatography of, and catalytic hydrogenation of, their 2,4-dinitrophenylhydrazones.

Sulphite was detected in the perfusate by the zinc nitroprusside reaction. It was not possible to prove that sulphite and sulphide were intermediates by reperfusing these substances through enriched soils because of their autoxidation to sulphate.

Sulphide could not be detected in the soil, the solution, or in the gases emanating from the system.

The same spectrum of amino acids was found with all the soils studied in the perfusion units, and for the soil *in situ*.

IV. Discussion

The identification of cysteinesulphinic acid and cysteic acid in the perfusate by paper chromatography, and the immediate oxidation of these two compounds when perfused through enriched soils strongly suggests that they were intermediates in the transformation of cysteine to sulphate.

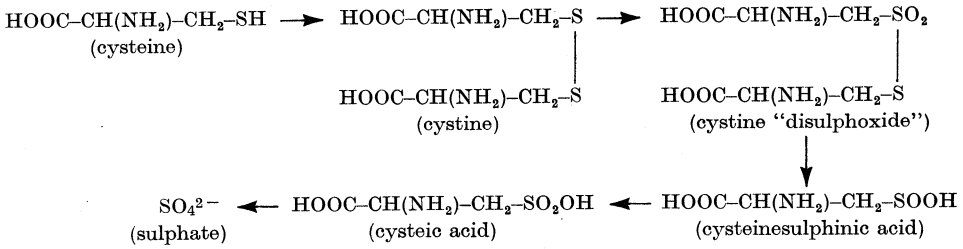
It is logical to assume that, in the perfusing system, cysteic acid was formed directly from cysteinesulphinic acid by oxidation. Kearney and Singer (1953*a*, 1953*b*) showed that this oxidation can occur in cell-free extracts of *Proteus vulgaris*, but they inferred that only a small amount of cysteinesulphinic acid was converted to sulphate via cysteic acid. Singer and Kearney (1955) concluded that the main pathway for the catabolism of cysteinesulphinic acid was via a transamination reaction with either α-ketoglutaric acid or oxaloacetic acid to form β-sulphinylpyruvic acid, HOOC–CO–CH_2–SOOH. Desulphination of this substituted pyruvic acid would then yield pyruvic acid and sulphite; the latter being oxidized to sulphate. They also concluded that cysteic acid was catabolized through the medium of β-sulphonylpyruvic acid, HOOC–CO–CH_2–SO_2OH.

However, neither β-sulphinyl- nor β-sulphonylpyruvic acids could be detected in the perfusate by chromatography of the 2,4-dinitrophenylhydrazones, nor could they be detected as cysteinesulphinic acid or cysteic acid, respectively, when the mixed hydrazones were converted to the parent amino acids by catalytic hydrogenation. It is possible that these two substituted pyruvic acids are so transitory that derivatives could not be formed, and thus their presence could not be detected. Therefore, the possibility that cysteinesulphinic acid and cysteic acid were catabolized via these substituted pyruvic acids could not be eliminated.

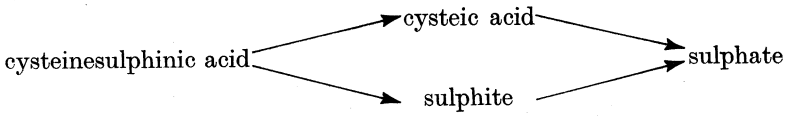
The presence of serine and its analagous keto acid, β-hydroxypyruvic acid, suggest that they are involved in the transformation under study. It seems unreasonable to expect that β-hydroxypyruvic acid should be formed in the system by side reactions when other keto acids (apart from the key metabolites pyruvic and α-ketoglutaric acids) were not detected. Desulphuration reactions yielding alanine described by Singer and Kearney (1955) would not explain the presence of serine and β-hydroxypyruvic acid in this system. The mechanism by which cysteinesulphinic acid and cysteic acids decompose to yield sulphite or sulphate, serine, and β-hydroxypyruvic is unknown.

That sulphide could not be detected in the system does not prove unequivocally that it was not involved in the transformation under study.

The results presented above, together with those obtained previously (Freney 1958), suggest that the *most probable* pathway for the oxidation of cysteine to sulphate in soil is:



It is possible that the overall reaction may involve a number of side or branched reactions, for example:



and that the whole process may be due to a number of different microorganisms.

Evidence to support the suggestion that cysteinesulphinic acid was formed directly from cystine "disulphoxide" was reported by Sweetman (1959), who found that this reaction occurred *in vitro*.

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