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Dissimilation of Methionine by Fungi[1]

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Soil fungi that attacked methionine required a utilizable source of energy such as glucose for growth. This is an example of co-dissimilation. Experiments with one of the fungi, representative of the group, are reported. In the absence of glucose, pregrown mycelium, even when depleted of energy reserves, oxidatively deaminated methionine with accumulation of α-keto-γ-methyl mercapto butyric acid and α-hydroxy-γ-methyl mercapto butyric acid. When glucose was provided, all of the sulfur of methionine was released as methanethiol, part of which was oxidized to dimethyl disulfide. No sulfate, sulfide, or hydrosulfide products were detected. Evidence was obtained that deaminase and demethiolase were constitutive. Deamination preceded demethiolation and α-keto butyric acid accumulated as a product of the two reactions. Other carbon residues were α-hydroxy butyric acid and α-amino butyric acid. Inability of the fungus to metabolize α-keto butyrate was responsible for its inability to utilize methionine as a source of carbon and energy. Several other fungi isolated from soil grew on α-amino butyrate but could not grow on methionine owing to inability to demethiolate it.

Methionine can be attacked by various microorganisms such as bacteria, including actinomycetes, and filamentous fungi (19, 26, 30), but it serves as a growth substrate for only exceptional microorganisms. Gram-negative, nonsporeforming, rod-shaped bacteria are the only cultures for which there is definite evidence of growth on methionine (13, 26). Most studies of methionine breakdown have been made with nongrowing washed cells, with cell-free extracts, or with cultures grown in media which contained other organic compounds as sources of energy.

Methanethiol is the generally reported sulfur product of methionine decomposition but several others have been noted. The bacteria of Segal and Starkey (26), which grew on methionine, deaminated the amino acid and then demethiolated it with production of methanethiol, part of which was oxidized to dimethyl disulfide.

This report is concerned with the dissimilation of methionine by fungi which were unable to use the amino acid for growth. Factors affecting their decomposition of methionine and the products of decomposition were determined. The results explain why most of the microorganisms which decompose methionine are unable to use it as a substrate for growth.

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MATERIALS AND METHODS

Cultures and cultural methods. The basal salts medium had the following composition: K_2HPO_4, 1 g; KH_2PO_4, 1.0 g; $MgCl_2 \cdot 6H_2O$, 0.5 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; $FeCl_3 \cdot 6H_2O$, 0.02 g; $ZnCl_2$, 0.02 g; distilled water to make 1,000 ml. The reaction was adjusted to *p*H 6.7. Solid media contained 30 g of agar per liter. The agar medium used to maintain the fungi contained the mineral salts plus 0.5% methionine and 1.0% glucose. Methionine (0.5%) was generally included in the solution media and additions of the following were made as indicated in the protocols of the experiments: glucose or other sugars, 1.0%; NH_4Cl, 0.5%; K_2SO_4, 0.05%. The sugars were sterilized separately.

The agar media used to recover fungi from methionine-enriched barnyard and forest soils (*p*H 7.8 and 3.5, respectively) contained 30 mg per liter each of rose bengal and streptomycin and 0.5% methionine or 1.0% glucose or both methionine and glucose. Generally, the fungi were cultivated in 100-ml portions of solution medium in 250-ml Erlenmeyer flasks incubated at 28 C on a rotary shaker. The inoculum consisted of 0.5 ml of a suspension of spores and mycelium removed from an agar slant in 7 ml of sterile water. For replacement cultures, the fungus was pregrown, in 100-ml portions of the basal medium containing 0.5% methionine and 1.0% glucose, for 4 days in shaken flasks. Cell material was filtered off aseptically, thoroughly washed with sterile distilled water, and resuspended in the test medium. Amount of growth is reported as the weight of washed fungus material after drying at 70 C for 24 hr.

Analytical methods. Methionine was determined by

two methods. One of these, the Lavine method (16), determines methionine which contains both amino and thioether groups. Thus, the method would give negative results when either group is absent. During bacterial dissimilation of methionine by a bacterium, deamination preceded demethiolation (26). It is concluded that this occurred with the fungus also because, in the results to be reported, the amount of amino nitrogen lost during dissimilation was nearly the same as the amount of methionine lost according to the Lavine method (Table 1). Furthermore, the amount of demethiolation consistently exceeded the loss of methionine according to the Lavine method (Tables 1 and 2 and Fig. 1). Therefore, results obtained by this method are generally designated deamination of methionine. The second method is the following modification of the method of McCarthy and Sullivan (12) for determining the thioether group: 1 ml of the test solution was mixed with 0.2 ml of 3% glycine, 0.4 ml of 2% sodium nitroprusside, and 0.2 ml of 1 N NaOH. The tubes were incubated in a water bath at 35 to 40 C for 5 to 10 min and then chilled in an ice bath for 2 min. To each tube was added 1 ml of a 1:9 mixture of HCl and H_3PO_4, and the tube was shaken vigorously for 1 min and cooled in water. A Beckman spectrophotometer was used to measure the optical density at 530 nm. The results obtained by this method are referred to as demethiolation of methionine.

To determine volatile sulfur products, a stream of air or oxygen was passed through the culture solution, held at 28 C, into two traps in series held in an ice bath. The first was 5% mercuric acetate which recovered methanethiol, and the second was 2% $HgCl_2$ which trapped dimethyl disulfide. The mercury precipitates were removed by filtration, washed, dried over $CaCl_2$, and used for the following determinations. Methanethiol in both the precipitate and in the solution of mercuric acetate was determined by the micro method of Sliwinski and Doty (28), modified to use a final volume of 10 ml. The method was standardized with pure $(CH_3S)_2Hg$ dissolved in 5% mercuric acetate. To determine dimethyl disulfide, weighed amounts of the precipitate formed with $HgCl_2$ were acidified with 6 N HCl in a closed vessel under a stream of nitrogen gas. When the solution was warmed in a water bath, methanethiol was evolved (5). The gas was recovered in a solution of mercuric acetate held in an ice bath. The methanethiol in the clear solution was determined as above.

Total amount of keto acid was determined by Friedemann's double-extraction method (9). Standard curves were prepared from α-keto butyric acid. Keto acids which accumulated in the culture solution were identified as their 2,4-dinitrophenyl hydrazones. The hydrazones were extracted from the medium with ethyl acetate and then reextracted with 10% Na_2CO_3. The alkaline solutions were acidified with HCl and the hydrazones were reextracted with ethyl acetate. The solvent was evaporated and the residue was dissolved in hexane. This solution was fractionated by adsorption on a silica gel column followed by elution with benzene and mixtures of benzene and increasing concentrations of methanol. The fractions were dried and analyzed by paper chromatography using the solvents indicated in the protocols of the experiments.

Tests for other organic acids were made on the aqueous solution remaining after extraction of the keto acids as the 2,4-dinitrophenyl hydrazones. The solution was made alkaline with NaOH, evaporated to 5 ml, acidified with H_2SO_4, adsorbed on a Celite column, and eluted with ether. The ether eluate was dehydrated with anhydrous Na_2SO_4 and evaporated under vacuum. The dry residue was analyzed by paper chromatography. Sulfur-containing acids were developed with Feigl's solution, and other acids were developed with aniline-xylose (2) or with a mixture of *p*H indicators and alkaline $KMnO_4$ (23). Ammonium salts were developed by spraying with Nessler's reagent. Keto acids were prepared from their amino acids according to Meister (17) but crystalline D-amino acid oxidase was used instead of kidney acetone powder.

Dubin's method (8) was used to determine α-amino nitrogen; the yellow color of the 2,4-dinitrofluorobenzene derivative of methionine was read at 340 nm. Absorption was maximal at this wavelength. The values were corrected for ammonia, when present, which was determined by Nesslerization. To identify amino acids, the filtered culture solution was deionized by the method of Thompson, Morris, and Gering (31) and analyzed by paper chromatography using the following two solvents: (a) water-saturated phenol which contained 0.002% 8-hydroxy quinoline; (b) butanol-acetic acid-water, 250:60:250 (2). Sugars were estimated by the anthrone reaction. Hexoses and disaccharides were determined by the method of Dimler et al. (7) and pentoses by that of Gary and Klausmeier (10).

Total sulfur content of dried cell material and dried culture solution was determined as sulfate after oxidation with sodium peroxide in a Parr bomb. The sulfate was precipitated with $BaCl_2$ and determined by means of a Klett nephelometer. Sulfate in the culture solution was determined also as the barium salt. Hydrosulfide was detected with nitroprusside (11).

RESULTS

Isolation of methionine-decomposing fungi. The abundance of fungi in methionine-enriched forest and barnyard soils was determined by plating on a medium in which methionine was the only source of carbon, nitrogen, and sulfur. Other media contained glucose (1.0%), NH_4Cl (0.5%), and K_2SO_4 (0.05%) in various combinations with and without methionine. Several thousand fungi per gram of soil were indicated by counts on the media which contained glucose and in which methionine was the only source of sulfur or nitrogen or both. There was meager development on media in which methionine was the only organic nutrient. Numerous colonies failed to grow when transferred to slants of basal agar medium with methionine as the only source of carbon, nitrogen, and sulfur, but they developed well when transferred to slants of a similar medium containing glucose. Several of the fungi were used in the following experiments, but the

results obtained with only one, *Aspergillus* sp., RS-la, are reported since these results are representative of the group.

Decomposition of methionine. When cultivated in basal salts medium with 0.5% methionine and 1.0% glucose, the fungus grew well, decomposed nearly all of the glucose in 3 days, deaminated about two-thirds of the methionine, and demethiolated 41% of it (Table 1). The value for loss of amino nitrogen determined by Dubin's method was nearly the same as that for loss of methionine determined by the Lavine method which was interpreted as deamination. Since the loss of methionine according to the Sullivan method (demethiolation) was less than that determined by the Lavine method as well as loss of amino nitrogen, it appears that deamination was more rapid than demethiolation and preceded it. No sulfate was produced. The increase in acidity is ascribed to production of organic acids from glucose. Results obtained with several fungi indicated that the amount of decomposed methionine was not proportional to the amount of growth; the culture which grew least was one of the most active decomposers of methionine. In another experiment, in which the inoculum consisted of a small amount of spore-mycelium mixture, there was increase in growth, loss of amino nitrogen, and loss of methionine with increase in the concentration of glucose from 0.01 to 1.0%. In medium which contained 1.0% glucose, 99% of the amino acid was deaminated and 56% was demethiolated. Since values for amino nitrogen by Dubin's method and for methionine by Lavine's method were nearly the same, only the latter results are reported in the following experiments. These values are designated deamination of methionine.

Influence of endogenous and exogenous energy sources on dissimilation. One portion of pregrown mycelium was suspended in basal medium containing methionine but lacking other organic compounds. An equal amount was suspended in distilled water and shaken 24 hr at 28 C to deplete the mycelium of stored energy material. The cell material was then removed and resuspended in methionine medium. Deamination was not affected by depletion of the endogenous reserves, whereas demethiolation was reduced. Furthermore, the amount of deamination greatly exceeded that of demethiolation; more than 90% of the methionine was deaminated in 4 days by both the depleted and nondepleted mycelium, whereas only 11 and 29% was demethiolated by the depleted and nondepleted mycelium, respectively.

Equal portions of similarly pregrown mycelium depleted of reserves were suspended in solution media containing methionine (0.5%) and methionine (0.5%) plus glucose (0.5%). Glucose had negligible effect on deamination but increased demethiolation (Fig. 1). Additional experiments indicated that the amount of demethiolation increased with glucose concentration up to 3%. The weight of the mycelium decreased during incubation in media lacking glucose or its equivalent and with concentrations of glucose up to 0.1%.

Pregrown depleted mycelium was homogenized in a Waring Blendor for 30 sec and 5-ml portions were added to 20-ml portions of distilled water containing 2 mg of methionine and 0, 0.20, or

TABLE 1. *Dissimilation of methionine*

Incubation period	pH	Dry wt of mycelium	Glucose consumed	Loss of amino N	Loss of methionine: Deamination	Loss of methionine: Demethiolation
days		*mg/100 ml*	%	%	%	%
1			34		12	16
3			95		44	30
5			100		48	31
7	4.9	131	100	67	63	41

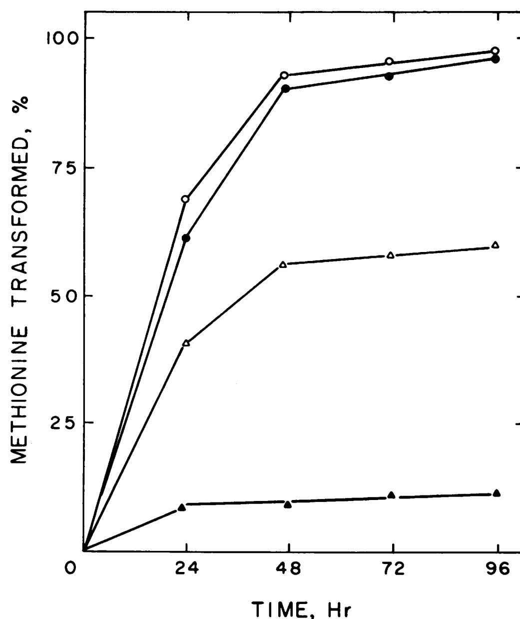

FIG. 1. *Effect of glucose on deamination and demethiolation by pregrown fungus mycelium. Deamination in presence (○) and absence (●) of glucose; demethiolation in presence (△) and absence (▲) of glucose.*

1.00 mg of glucose. During incubation, a stream of oxygen was passed through the culture solution into mercuric acetate held in an ice bath to trap methanethiol. Periodic determinations indicated that methanethiol was produced only when glucose was available (Fig. 2). On adding glucose after all had been consumed, more methanethiol was produced.

No methanethiol was produced from methionine when the cell suspension was sparged with nitrogen or carbon dioxide, whereas it was produced when the sparging gas was oxygen or air. Considerably more methanethiol was produced when oxygen was used.

To determine whether other compounds could substitute for glucose as energy sources, pregrown depleted mycelium was suspended in basal salts medium containing 0.2% methionine and 0.5% of the carbohydrate and incubated as shaken cultures for 4 days. The amount of mycelium which was added to each flask had an equivalent dry weight of 112 mg. The fungus grew on all the sugars but lost weight in the medium which contained no sugar (Table 2). Most of the methionine was deaminated in all media, even in the one which contained no carbohydrate, but the amount of demethiolation differed and was small in the absence of an energy source.

Constitutive nature of capacity to degrade

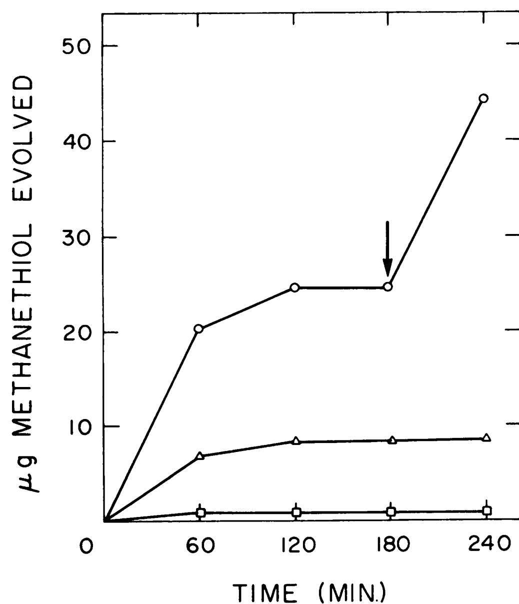

FIG. 2. *Effect of glucose [0.004% (○), 0.0008% (△), none (□)] on production of methanethiol by pregrown fungus mycelium in 25 ml of culture solution. At arrow, a second increment of 1 mg of glucose was added.*

TABLE 2. *Influence of some carbohydrates on breakdown of methionine by pregrown mycelium*

Substrate	Loss of substrate	Dry wt of mycelium[a]	Loss of methionine	
			Deamination	Demethiolation
	%	mg	%	%
None		83	94	8
Glucose	100	255	92	54
Fructose	100	291	97	69
Galactose	100	298	96	72
Arabinose	46	220	97	44
Lactose	40	243	90	43
Maltose	87	257	92	65

[a] Initial weight, 112 mg.

methionine. Fungus mycelium was grown in media which contained 1.0% glucose and one of the following: (a) 0.5% methionine, (b) 0.5% NH_4Cl and 0.05% K_2SO_4, (c) 0.5% asparagine and 0.05% K_2SO_4. The three lots of mycelium were washed and homogenized, and equal amounts were suspended in 25-ml portions of distilled water containing 2.5 mg of glucose and 5 mg of methionine. Determinations for methanethiol were made at short intervals (30 min) to ascertain whether there was an initial short lag phase of demethiolation. The course of production of methanethiol was the same irrespective of presence of methionine in the growth medium and it was nearly linear for the 2-hr incubation period. Another experiment indicated that both deamination and demethiolation by pregrown mycelium were unaffected by lack of methionine in the growth medium.

In various experiments, washed mycelium pregrown in media which contained glucose and methionine failed to grow in methionine media which lacked glucose or its equivalent. Actually, the mycelium lost weight when incubated in these replacement media for 1 week.

Sulfur balance and identification of volatile products. Pregrown washed cell material was suspended in 100 ml of basal medium which contained 0.5% methionine and 2.0% glucose. The solution was incubated as a shaken culture for 4 days. Sterile air was passed through the medium and into solutions of 2% mercuric acetate and 2% $HgCl_2$ to trap methanethiol and dimethyl disulfide. Methionine content of the culture solution was determined initially and at the end of the experiments by the method of McCarthy and Sullivan. Nearly half of the methionine was demethiolated and practically all of the sulfur which was released was recovered as the volatile products, methanethiol and dimethyl

disulfide (Table 3). A small amount of sulfur was contained in the cell material which was produced during the experiment. Most of the released sulfur was recovered as dimethyl disulfide but larger amounts were released as methanethiol in other experiments. In two experiments carried out for purposes other than to determine sulfur balances, 100% and 49% of the released sulfur was methanethiol. Means of identification of the products have been described previously (5, 25). When the precipitate which had been trapped in the $HgCl_2$ was warmed with 5 N NaOH under N_2 and the released gases were bubbled through 2% $HgCl_2$, no precipitate was formed, which indicated absence of dimethyl sulfide. Neither sulfide nor hydrosulfide compounds were detected as products.

Acids produced from methionine. Pregrown depleted mycelium was suspended in basal salts media which contained the following: (a) 1.0% glucose and 0.5% NH_4Cl, (b) 1.0% glucose and 0.5% methionine, (c) 0.5% methionine. After the cultures had incubated for 2 days, the 2,4-dinitrophenyl hydrazones of the keto acids in the culture solutions were prepared and analyzed by paper chromatography using the following solvents: (a) butanol-water-ethyl alcohol, 5:4:1; (b) methanol-benzene-butanol-water, 4:2:2:2 (18). Hydroxy phenyl pyruvic acid was found in both media which contained glucose irrespective of the presence of methionine, and phenyl pyruvic acid was detected in the glucose medium which contained no methionine (Table 4). It is concluded from this that they originated from glucose. In both culture solutions which contained methionine, α-keto-γ-methyl mercapto butyric acid (α-keto methionine) was present, and there was more in the methionine medium which contained no glucose. In the culture solution which contained both methionine and glucose, there was α-keto butyric acid. These results support the concept that α-keto methionine was the product of deamination of methionine and that it was demethiolated to α-keto butyric acid.

TABLE 3. *Sulfur balance of decomposed methionine*

Determination	Sulfur content	
	Amt	Per cent of initial S
	mg	
Initial medium		
Methionine	111.6	100
Culture solution		
Total	52.2	47
Methionine	50.6	
Other	1.6	
Volatile products	53.1	48
Methanethiol	8.6	
Dimethyl disulfide	44.5	
Mycelium	2.9	3

After extracting keto acids from the culture solution which had contained methionine and glucose, determinations were made for other acids by paper chromatography using the following as solvents: solvent a of the preceding experiment; (c) ethyl alcohol-concentrated NH_4OH–water, 80:5:15; (d) butanol–acetic acid–water, 250:60: 250 (2); (e) ethyl alcohol-water–concentrated NH_4OH, 95:5:1 (14). Both α-hydroxy-γ-methyl mercapto butyric acid (α-hydroxy methionine) and α-hydroxy butyric acid were found, but only a small amount of the latter. Tests for amino acids indicated the presence of only α-amino butyric acid. There was no homoserine. Pregrown fungus cells produced α-hydroxy butyric acid from α-hydroxy methionine.

Influence of pH on dissimilation. Equal amounts of pregrown washed mycelium were suspended in glucose, methionine, mineral salts media adjusted to reactions from *p*H 4.5 to 9.0 and incubated for 4 days. Over this range of reaction there were only slight differences in the amounts of deamination and demethiolation. Minimum and maximum values for deamination were 82 and 100%, respectively, and those for demethiolation were 51 and 59%. Deamination decreased gradually with increase in *p*H. The *p*H of media that were alkaline initially decreased.

Carbon residues of methionine dissimilation and their effect on growth. Since methionine failed to support growth, it seemed likely that its deaminated and demethiolated products could not be metabolized. This conclusion was supported by detection of organic products of dissimilation in culture solutions (Table 4). Furthermore, the fungus was unable to grow on α-amino butyrate, but both it and methionine served as sources of nitrogen for growth in glucose-containing media.

Additional evidence that α-amino butyric acid was deaminated and that the resulting keto acid was not metabolized is shown in Fig. 3. Equal amounts of pregrown washed mycelium were suspended in 20 ml of 0.05 M phosphate buffer (*p*H 7.2) containing 4.12 mg of α-amino butyric acid (2 mM) and in two other portions of buffer containing equivalent amounts of α-keto butyric acid, one adjusted to *p*H 4.5 and the other to *p*H 7.5. The added keto acid was unaltered by the fungus even at *p*H 4.5 which would have favored its penetration into the mycelium. The keto acid produced by deamination of the amino acid accumulated. This was identified as α-keto butyric acid by paper chromatography.

TABLE 4. *Keto acids produced from glucose and methionine*

Additions to basal medium	Fraction of keto acid DNPH[a]	R_F Solvent a	R_F Solvent b	Identification
Glucose + NH_4Cl	1	0.73	0.85	Phenyl pyruvic acid
	2	0.88	0.95	Hydroxy phenyl pyruvic acid
Glucose + methionine	3	0.62	0.82	α-Keto butyric acid
	4	0.81	0.88	α-Keto methionine
	5	0.86	0.96	Hydroxy phenyl pyruvic acid
Methionine	6	0.81	0.88	α-Keto methionine
				Authentic compounds
		0.77	0.86	Phenyl pyruvic acid
		0.90	0.93	Hydroxy phenyl pyruvic acid
		0.64	0.85	α-Keto butyric acid
		0.81	0.88	α-Keto methionine

[a] 2,4-Dinitrophenyl hydrazones.

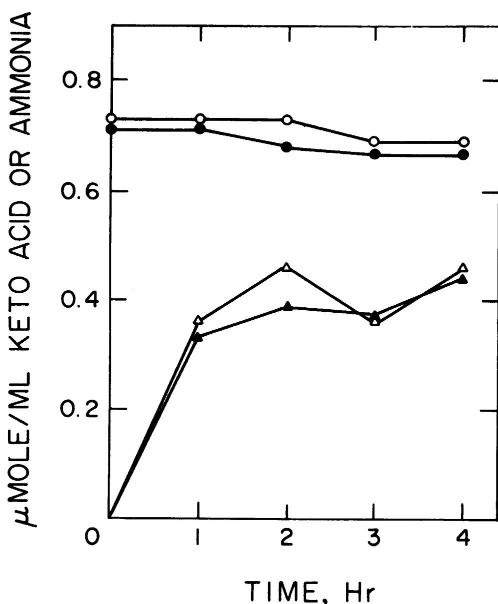

FIG. 3. *Influence of pregrown fungus mycelium on α-keto butyric acid at pH 4.5 (○) and pH 7.2 (●) and on α-amino butyric acid with production of ammonia (△) and α-keto butyric acid (▲).*

In another experiment, the fungus was inoculated into media of *p*H 4.5 which contained the organic compounds (0.05 M) listed in Table 5, NH_4Cl, and K_2SO_4. The cultures were incubated for 4 days. Growth was limited though significant on pyruvate, propionate, and succinate.

DISCUSSION

Whereas certain bacteria use methionine as a growth substrate (13, 26), search for filamentous fungi able to do this failed. Therefore, it is concluded that fungi lack the capacity to grow at the expense of methionine or that this capacity is a rare endowment, as yet undetected. Ability to decompose methionine in the presence of growth-supporting organic compounds such as glucose is a property of many fungi recovered from soil as well as of other fungi (6, 29, 32). This ability was noted also by Uchida (33) for *Shigella flexneri*. The capability to decompose a compound in the presence of another compound which supports growth, but not in the absence of the latter compound, is designated co-dissimilation.

When provided glucose, representative fungi deaminated and demethiolated the amino acid. With small amounts of spores and mycelium as inoculum, both deamination and demethiolation increased with increase in the glucose concentration, whereas, with replacement cultures using pregrown mycelium, methionine was attacked in the absence of glucose or its equivalent. Methionine was only deaminated, and the cells lost weight during the incubation. Although no energy source was required for deamination, a source of energy was needed for demethiolation; in absence of glucose, demethiolation was limited,

TABLE 5. *Influence of some potential intermediates on growth*

Compound	Dry wt of mycelium
	mg
Glucose	303
Pyruvate	17
Propionate	40
Succinate	26
Methyl malonate	0

particularly by pregrown mycelium depleted of endogenous reserves. Oxygen was required for demethiolation. Likewise, decomposition of methionine by a methionine-decomposing bacterium was promoted by aeration (26). However, others (13, 19–22) reported that certain facultative anaerobic bacteria and their enzymes demethiolated methionine anaerobically, and evidence was provided (21, 22) that demethiolation was more rapid in the absence of oxygen.

Deamination and demethiolation by pregrown mycelium were unaffected by absence of methionine in the growth medium, which indicates that the amino acid is not required for synthesis of deaminase and demethiolase; that is, the enzyme systems are constitutive. To the contrary, a bacterial culture which was unable to grow on methionine initially, was able to do this after being grown in a medium which contained both methionine and glucose (26).

Methionine was first deaminated and then demethiolated with release of the sulfur as methanethiol, part of which was oxidized to dimethyl disulfide (Fig. 4). This is the same course of events noted by Kallio and Larson (13) and Segal and Starkey (26) for the initial stages of dissimilation of methionine by bacteria. No other sulfur products were detected. Others reported that methanethiol, dimethyl disulfide, and related compounds were products of methionine breakdown by several fungi: methanethiol and ethyl sulfide by *Oidium lactis* in presence of sucrose (1), methanethiol and dimethyl sulfide by *Scopulariopsis brevicaulis*, and methanethiol by *Schizophyllum commune* on bread media (6), by *Microsporum gypseum*, *S. brevicaulis*, and *Aspergillus niger* with glucose (29), by *Penicillium caseicolum* on skim milk and casein (32), and by diverse other fungi in a medium which contained glucose and peptone (26).

The principal carbon residue of methionine

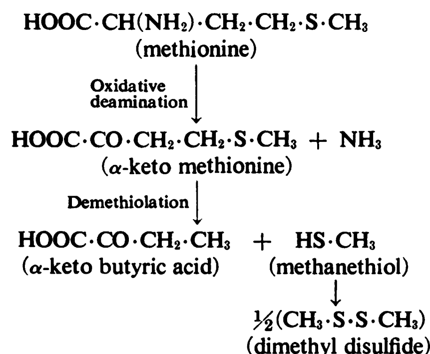

FIG. 4. *Deamination and demethiolation of methionine.*

dissimilation in the absence of glucose was α-keto methionine, the product of an amino acid oxidase. This keto acid was identified as the product of methionine transformation by animal tissue slices (3, 4) and by an amino acid oxidase of *Trigonopsis variabilis* (27). In the presence of both glucose and methionine, α-keto butyric acid accumulated. Both keto acids were the anticipated products of oxidative deamination and of demethiolation with production of methanethiol. In addition, α-hydroxy methionine and α-hydroxy butyric acid were detected. Whether these are direct products of deamination and demethiolation or of reduction of the keto homologues is unknown. Some α-amino butyric acid which was detected may have originated by demethiolation of methionine. Others (1, 13, 20, 21, 34) identified α-keto butyric acid as the product of demethiolation of methionine by bacteria and their enzymes. Even though methionine, α-amino butyrate, and α-keto butyrate failed to support growth, the fungus grew on propionic acid and pyruvic acid which might be expected to be produced from them (15, 24). Therefore, it is unlikely that these acids were generated from the products of demethiolated methionine.

Although the fungi which were tested were unable to oxidize α-keto butyrate, several fungi isolated from soil could use α-amino butyrate as their sole source of carbon and nitrogen. However, they could not use methionine as an energy source and did not produce volatile sulfur products from it. Their inability to develop on methionine is ascribed to lack of demethiolase.

The results lead to the following conclusions. Various fungi attack methionine, deaminating and demethiolating it, leaving α-keto butyric acid as the principal carbon skeleton. This cannot be metabolized. Therefore, other organic compounds are required as energy sources. Many microorganisms, including diverse bacteria and actinomycetes as well as fungi, attack methionine but cannot grow on it. For them, as for the fungi studied, this is probably due to inability to metabolize the deaminated and demethiolated carbon residues.

ACKNOWLEDGMENT

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