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Dissimilation of Methionine by *Achromobacter starkeyi*[1]

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Methionine was decomposed by some bacteria which were isolated from soil. The sulfur of the methionine was liberated as methanethiol, and part of this became oxidized to dimethyl disulfide. Detailed studies with one of these cultures, *Achromobacter starkeyi*, indicated that the first step in methionine decomposition was its oxidadative deamination to α-keto-γ-methyl mercaptobutyrate by a constitutive amino acid oxidase. The following steps were carried out by inducible enzymes, the synthesis of which was inhibited by chloramphenicol. α-Keto-γ-methyl mercaptobutyrate was split producing methanethiol and α-keto butyrate which was oxidized to propionate. The metabolism of propionate was similar to that described for animal tissues; the propionate was carboxylated to succinate via methyl malonyl coenzyme A, and the succinate was metabolized through the Krebs cycle.

From studies of the decomposition of methionine by bacteria, mostly of the genus *Pseudomonas* (15, 23-26, 30, 35), it was concluded that the principal products are the volatile sulfur compounds, methanethiol and dimethyl disulfide, and α-keto butyric acid which is further decomposed and can serve as the source of carbon and energy for growth. Fungi produced the same sulfur compounds and α-keto butyrate but they were unable to utilize methionine as a source of carbon and energy because they could not transform α-keto butyrate (27-29).

Cleavage of the C-S bond of the amino acid with production of α-keto butyrate follows the oxidative deamination. Subsequent events are speculative; no study has been published of the course of events during degradation of the butyrate.

This report is concerned principally with the dissimilation of methionine by one of several methionine-decomposing bacteria isolated from soil, identification of the sulfur products, and the establishment of the series of reactions through which α-keto butyrate is dissimilated.

MATERIALS AND METHODS

Cultures and cultural methods. Both untreated soils and soils enriched with methionine (1.0%) were plated out on a basal salts agar medium which contained 0.5% methionine as the only organic nutrient (28). Many bacterial cultures were isolated from these plates and tested for their ability to decompose methionine. The most active strain, NE-2, was identified as *Achromobacter* sp. by the method of Skerman (32), and it has been described as a new species, *A. starkeyi* (Ruiz-Herrera, Antonie van Leewenhoek J. Microbiol. Serol., *in press*).

The liquid medium containing 0.5% methionine was similar to one described previously (28) but it differed in that the *p*H was 7.2. For some experiments, 0.05% yeast extract (Difco) was included in the medium because it shortened the lag period of growth. In some media glucose (1%), alanine (0.5%), and K_2SO_4 (0.05%) were used in place of methionine. The culture was inoculated into 100-ml portions of culture medium in 250-ml Erlenmeyer flasks which were incubated at 27 C on a rotary shaker (250 cycles/min). For experiments with washed cells, the culture solutions were centrifuged; the recovered cells were washed three times with 0.05 M phosphate buffer (*p*H 7.3) and finally diluted with an amount of buffer sufficient to provide a suspension with 1.4 mg (dry weight) of cells per ml for all experiments.

Oxygen uptake was measured by a Bronwill Warburg respirometer at 28 C by standard techniques (34). Cell-free extracts were prepared from cells which were disrupted by means of a Ribi cell fractionator. The extracts were centrifuged in the cold at 10,000 × *g* for 30 min, and the supernatant served as the crude enzyme extract. Formation of ammonia (9) or keto acid (27) from methionine or α-keto-γ-methyl mercaptobutyric acid (α-keto methionine) served as evidence of deamination. The term demethiolation refers to liberation of methanethiol re-

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sulting from cleavage of the C-S bond of methionine and related compounds. It was determined by means of Conway units as previously described (29). Formation of keto acid from DL-methionine by cell-free extracts served as evidence of oxidation of methionine. For this determination, 1 ml of crude cell extract was added to 2 ml of a solution containing 2.5 μmoles of DL-methionine, 1.5 μmoles of tris(hydroxymethyl)-aminomethane (Tris) buffer (*p*H 7.3), 3 μmoles of ethylenediaminetetraacetic acid (EDTA), and 0.3 μmole of mercaptoethanol. After 1 hr, 0.5 ml of 50% trichloroacetic acid was added, the precipitate was removed, and the amount of keto acid in the supernatant was determined.

Oxidation of α-keto butyrate was determined by measurement of the $^{14}CO_2$ liberated from α-amino butyrate-*1*-^{14}C by cell-free extract incubated in the presence of D-amino acid oxidase. The reaction mixture (1.8 ml) contained 1.0 ml of cell-free extract, 150 μmoles of Tris buffer (*p*H 7.3), 40 μmoles of $MgCl_2$, 1.6 μmoles of lipoic acid, 1.6 μmoles of nicotinamide adenine dinucleotide (NAD), 0.8 μmole of diphosphothiamine (DPT), 0.5 mg of D-amino oxidase, 1 mg of crude catalase, and 0.4 μmole of α-amino butyrate-*1*-^{14}C with a specific activity of 0.25 μCi/μmole. Incubation was carried out in Conway units in which the $^{14}CO_2$ was trapped in 0.5 ml of 50% KOH held in the center chamber. After 1 hr, carrier K_2CO_3 and $BaCl_2$ were added to the KOH. The precipitate was recovered and washed, and its radioactivity was determined.

Propionate activation was determined by the method of Berg (4) for measurement of yeast acetocoenzyme A-(CoA)kinase. The reaction mixture consisted of 0.8 ml of cell-free extract, 100 μmoles of phosphate buffer (*p*H 7.3), 10 μmoles of $MgCl_2$, 10 μmoles of adenosine triphosphate (ATP), 50 μmoles of NaF, 20 μmoles of reduced glutathione, 10 μmoles of sodium propionate, 0.2 μmole of CoA-SH, and 200 μmoles of hydroxylamine. The final volume was 2.0 ml. After 1 hr, 4 ml of acid $FeCl_3$ was added, the mixture was centrifuged, and absorbancy per cm of the supernatant fluid at 540 nm was measured by a Beckman DU spectrophotometer.

Carboxylation of propionyl-CoA was measured as described by Lane and Halenz (18). The 4 ml of reaction mixture contained cell-free extract, 150 μmoles of Tris buffer (*p*H 7.4), 6 μmoles of $MgCl_2$, 15 μmoles of reduced glutathione, 12 μmoles of ATP, 30 μmoles of KCl, 1 μmole of propionyl-CoA (or 1 μmole of sodium propionate and 1 mg of CoA-SH), and 0.1 ml of $NaH^{14}CO_3$ with an activity of 50,000 counts/min. After 1 hr, carrier $NaHCO_3$ was added followed by 0.3 ml of concentrated H_2SO_4, and the tubes were held in a boiling-water bath for 10 min. After centrifugation, the supernatant fraction was extracted with ether in a liquid-liquid continuous extractor. The ether layer was removed and evaporated; and the residue was dissolved in water, transferred to planchets, and evaporated; and its radioactivity was determined.

Succinic dehydrogenase was determined by the method of Ells (10) by using 3.0 ml of the following reaction mixture in 3 ml absorption cells with a 1-cm light path: 150 μmoles of phosphate buffer (*p*H 7.3), 0.45 μmole of 2,6-dichloroindophenol, 0.15 mg of phenazine methosulfate, 30 μmoles of KCN, and 6 μmoles of sodium succinate. The reaction was initiated by addition of 0.1 ml of cell-free extract (1.5 mg of protein). Change in absorbancy (Δ OD) was followed in a Beckman DU spectrophotometer coupled to a Photovolt Varicord recorder.

When methyl malonyl CoA mutase was measured by the method of Beck (1), there was interference from high levels of succinic dehydrogenase in the crude cell extract. Therefore, it was determined indirectly, substituting 1.2 μmoles of methyl malonyl CoA for the 6 μmoles of succinate in an incubation mixture similar to that used to determine succinic dehydrogenase. The reaction was started by addition of 0.2 ml of cell-free extract. Change in absorbancy per cm was recorded at 600 nm.

Analytical methods. Determination of keto acids, sulfate, and methanethiol, and separation of methanethiol and dimethyl disulfide from incubation mixtures was carried out as previously described (28). Loss of methionine determined by the method of Lavine (20) is referred to as methionine deamination; loss determined by the modified method of Hess and Sullivan (14) is referred to as demethiolation. Production of methanethiol served as the index of breakdown of thioethers. Methanethiol was determined by a method similar to that previously described (29), by using Conway dishes. The outer chamber contained 0.5 ml of cell suspension (6 mg, dry weight) and 0.5 ml of a 20 mM solution of the substrate. The center well contained 1 ml of 5% mercuric acetate. The units were incubated at 28 C. To stop the reaction, 0.1 ml of 80% trichloroacetic acid was injected through the rubber stopper into the outer chamber. After being held for 1 hr at 37 C to trap all of the methanethiol, the thiol in the mercuric acetate was determined.

Ammonia was determined by the Conway microdiffusion technique (9). The ammonia was titrated with 0.02 N H_2SO_4. Organic acids were separated from the acidified culture solution with ether in a continuous liquid-liquid extractor and analyzed by paper chromatography. Spots were developed as described previously (28). Protein was measured by the method of Lowry et al. (22), by using crystalline bovine serum albumin for the standard. Propionyl CoA was synthesized by the method of Simon and Shemin (31), and methyl malonyl CoA was prepared by the method of Trams and Brady (33); the concentration was measured by the hydroxyamate method of Lipmann and Tuttle (21). Methionine was used to prepare α-keto methionine as described previously (28). Radioactivity of dried samples was measured by a model 186A gas flow counter (Nuclear-Chicago Corp.). The radioactivity was corrected for self-absorption by extrapolation to infinite thinness.

RESULTS

Methionine dissimilation by growing bacterial cultures. Twelve cultures isolated from soil were cultivated for 10 days in the basal medium in which methionine was the only source of carbon,

nitrogen, and sulfur. Residual nitrogen and products of its breakdown were determined at 48-hr intervals. Most cultures had long lag phases. Table 1 shows that several cultures decomposed most of the methionine. Loss of the amino acid as measured by the amounts of deamination and demethiolation was similar; there was, however, somewhat more deamination than demethiolation. High levels of growth were associated with high levels of methionine decomposition. Neither sulfate, thiosulfate, nor polythionates was detected in any of the culture solutions. All of the solutions developed a strong odor of methanethiol.

We reported previously (27–28) that the enzymes involved in methionine breakdown by *Aspergillus* sp. (identified as *A. flavus*) were constitutive. Results obtained with the bacterial cultures showed that, although deamination was brought about by a constitutive enzyme system, demethiolation was effected by an inducible enzyme. The demethiolase was induced by D- and L-methionine and by ethionine. The following failed to induce demethiolation: *S*-methyl-L-cysteine, γ-mercaptobutyrate, alanine, and glutamic acid.

Alanine was an excellent source of nitrogen for growth of the bacterium on glucose, ethionine was fair, and the following were poor: ammonia, nitrate, glutamic acid, and *S*-methyl-L-cysteine. L-Methionine was superior to D-methionine.

All of the following experiments were carried out with the culture of *A. starkeyi* which grew well in a medium in which methionine was the only organic compound. Figure 1 shows that it demethiolated several thioethers. Highest demethiolase activity was noted with α-keto methionine.

TABLE 1. *Decomposition of methionine by bacterial cultures isolated from soil*

Culture	Dry wt	Loss of methionine		NH_4^+_N
		Deamination	Demethiolation	
	mg/100 ml	%	%	*mg*
B3-1	108	100	96	12.1
F1-1	90	100	95	10.0
F1-2	85	100	97	9.2
NE-2	80	100	94	10.2
F1-3	70	100	95	10.4
NE-1	67	100	84	11.9
F2-1	13	17	15	0
B1-2	13	12	11	0.7
C1-2	10	9	14	0.2
C3-1B	10	15	8	0.1
B1-1	5	17	16	0
C2-1a	5	12	4	0

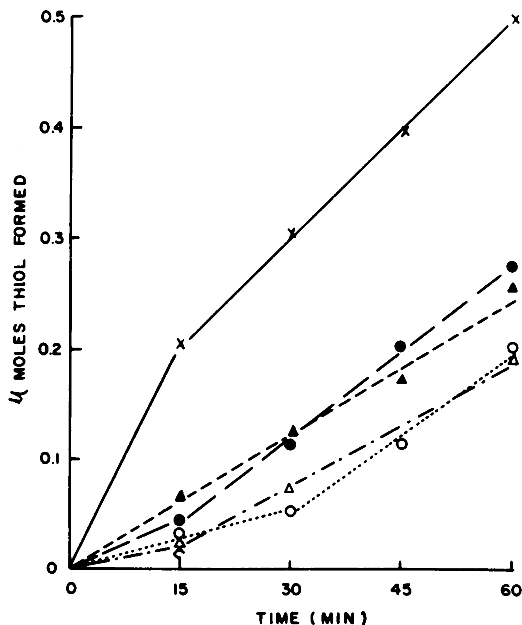


FIG. 1. *Decomposition of thioethers by A. starkeyi. Symbols:* ×, *α-keto methionine;* ○, *L-methionine;* ●, *D-methionine;* △, *α-hydroxy methionine;* ▲, *DL-ethionine.*

Both D-methionine and DL-ethionine were demethiolated rapidly, whereas L-methionine and α-hydroxy-γ-methyl mercaptobutyric acid (α-hydroxy methionine) were transformed more slowly.

Miwatani et al. (24) and Kallio and Larson (15) noted that more methionine was decomposed by *Pseudomonas* sp. under anaerobic conditions than in the presence of oxygen. We found (28) that oxygen was required by *Aspergillus* sp. to split the thioether bond of methionine. To test the effect of oxygen on demethiolation of methionine by washed cells of *A. starkeyi*, washed cells grown on a methionine medium were incubated with 10 mmoles of DL-methionine in a final volume of 10 ml of 0.05 M phosphate buffer (*p*H 7.3). Either nitrogen or oxygen was bubbled through the cell suspension. The effluent gas was passed through 5% mercuric acetate held in an ice bath to trap evolved methanethiol. Demethiolation occurred only when oxygen was present.

Demethiolation and deamination of methionine by washed cells. To identify the volatile sulfur compounds produced from methionine, a 9-ml suspension of washed cells (29 mg, dry weight) was incubated with 1.6 μmoles of DL-methionine (methyl labeled, 0.125 μCi/μmole) in 0.05 M phosphate buffer (*p*H 7.3). A stream of air was passed through the culture solution and then

through 5% mercuric acetate and 3% mercuric chloride to trap the released radioactive volatile sulfur compounds. Analysis of the trap solutions and results of other experiments showed that methanethiol was the principal sulfur product and that there were small amounts of dimethyl disulfide which appeared late in the incubation period. Similar results were obtained with another bacterium (30) and a fungus (28).

Demethiolation of DL-methionine was little affected by reaction from *p*H 5.5 to 9.0, but it was somewhat greater above neutrality. From *p*H 5.5 to 8.0, the amount of deamination was nearly the same but above *p*H 8.0 it decreased. On the molar basis, deamination was approximately four times as great as demethiolation.

Effect of inhibitors on breakdown of methionine. Sodium arsenite inhibited demethiolation 69% at a concentration of 10^{-3} M. Others (15, 24, 25) have reported also that arsenite inhibits breakdown of methionine by bacteria. Hydroxylamine, NaCN, and NaN_3 at 10^{-3} M had no inhibitory effect on either deamination or demethiolation of methionine. Iodoacetate, *p*-chloromercuribenzoate (PCMB), and semicarbazide at the same concentration did not affect deamination, but evolution of methanethiol was reduced 80% by iodoacetate and PCMB and 31% by 3×10^{-3} M semicarbazide. The same concentration of semicarbazide reduced oxygen uptake 53% when methionine was the substrate.

Initial reaction in breakdown of methionine. When 154 mg (dry weight) of cells was incubated with 10 mmoles of methionine in a solution containing 10^{-2} M semicarbazide, a keto acid was trapped as its semicarbazone. This was transformed into its 2,4-dinitrophenylhydrazone (DNPH) by addition of 0.1% dinitrophenylhydrazine in HCl, and the yellow compound was recrystallized from ethyl alcohol-water and dried over $CaCl_2$. The DNPH was analyzed by descending paper chromatography in water-saturated butyl alcohol (7) and butyl alcohol-ethyl alcohol-water (5:4:1; reference 5). Only one spot appeared and it had the same mobility as the DNPH of α-keto methionine.

The hydrazone was analyzed also by paper electrophoresis in 0.02 M Veronal buffer of *p*H 8.5. In 5 hr the material migrated to a single spot at the same distance from the cathode as the DNPH of α-keto methionine. The uncorrected melting points of the recrystallized derivatives of the unknown and of pure α-keto methionine were 150 to 153 C and 150 to 151 C, respectively. This evidence establishes the identity of the keto acid which was produced by cells inhibited by semicarbazide as α-keto methionine, and suggests that it is the first product of methionine breakdown.

Intermediate carbon compounds of methionine dissimilation. A 20-ml reaction mixture contained 110 mg of washed cells (dry weight), 400 μmoles of DL-methionine, and 0.05 M phosphate buffer (*p*H 7.3). After 2 hr the cells were spun down in the cold, 1.0 ml of concentrated H_2SO_4 was added to the supernatant fluid, and the volatile acids were collected by steam distillation, neutralized with KOH, and analyzed by paper chromatography with ethyl alcohol-water-concentrated NH_4OH (95:5:1; reference 17). Only one spot developed, and this had the same R_F value as propionic acid. Nonvolatile acids were adsorbed on a celite column, eluted with ether, and analyzed by paper chromatography with the solvent system just described. Four spots developed which had R_F values of 0, 0.02, 0.09, and 0.17. The spot which was the most strongly marked was that with R_F 0.09, and it was identified as α-keto butyric acid which had the same mobility. The spot with R_F 0.17 was similarly identified as α-hydroxy methionine.

The detection of propionic acid suggests that the α-keto butyric acid was oxidized to propionic acid which was metabolized by the same reactions effected by animal tissues. This involves carboxylation of the propionic acid to methyl malonate and its rearrangement to succinic acid (2, 3, 11–13, 16, 19). Therefore, evidence was sought about this supposition.

At *p*H 4.5 but not at *p*H 7.3, α-keto butyrate, propionate, and succinate were oxidized by washed cells without a lag phase. Methyl malonate was not oxidized, which was contrary to expectation. The effect of chloramphenicol (CAP) on oxidation of substrates by induced and noninduced cells was determined as follows. The culture was grown for 72 hr in the basal medium containing methionine to obtain induced cells and in a similar medium which contained glucose, alanine, and K_2SO_4 in place of methionine to obtain noninduced cells. The washed cells were suspended in 0.05 M phosphate buffer (*p*H 4.5) containing 2 μmoles of substrate. Figure 2 shows that CAP at 58 μg/ml did not inhibit oxidation by induced cells of either methionine, α-keto butyrate, or propionate but it did inhibit their oxidation by noninduced cells.

These results indicate that the enzymes involved in dissimilation of methionine following the step of deamination are induced and that their synthesis is blocked by CAP. It was noted also that the amounts of oxygen taken up by the cells in the oxidation of some substrates agreed well

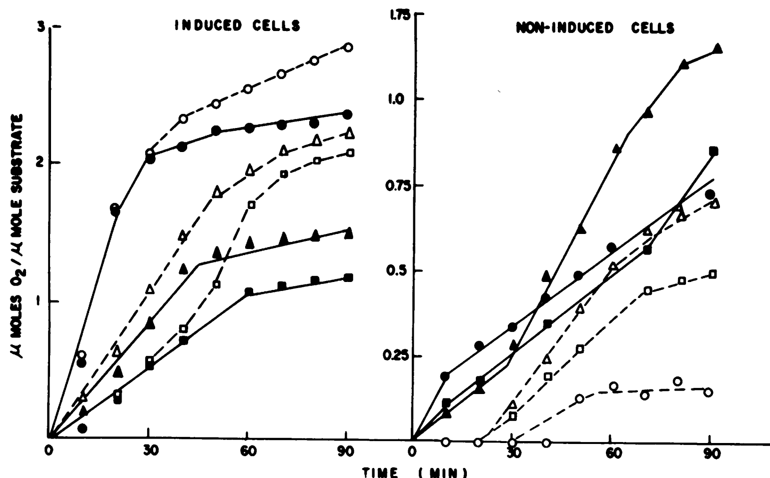


FIG. 2. *Effect of chloramphenicol on oxidation of substrates by induced and noninduced bacterial cells. Symbols:* ○, ●, *DL-methionine;* △, ▲, *α-keto butyric acid;* □, ■, *propionic acid. Closed symbols, no chloramphenicol; open symbols, 55 μg of chloramphenicol per ml.*

with the calculated oxygen uptake except for methionine (Table 2).

If succinic acid is an intermediate in methionine degradation, it would be expected that malonate would inhibit oxidation of the postulated intermediates, since it has been well established that it is a competitive inhibitor of succinic dehydrogenase. Cells used to test the effect of malonate were obtained by the same procedure as that used for the preparation of induced cells, used in the preceding experiment. The following radioactive substrates were introduced into the respiration vessels: 0.5 μCi/μmole of propionate-*1*-^{14}C, 0.25 μCi/μmole of α-amino butyrate-*1*-^{14}C. After oxygen uptake was determined for 3 hr, the contents of the center wells of the respiration vessels were removed, the walls were washed several times, and the washings were added to the withdrawn solution. Sufficient K_2CO_3 carrier to produce 10 mg of $BaCO_3$ was added to the solution followed by $BaCl_2$. The precipitated $BaCO_3$ was recovered by filtration, dried with acetone, glued into planchets; the radioactivity was then determined. No correction was made for self-absorption. Table 3 shows that malonate blocked oxidation of the five substrates. Cells of the bacterium in a solution containing methionine and 5 mM malonate were incubated for 1 hr, after which the acids were

TABLE 2. *Oxygen uptake by A. starkeyi in the presence of some substrates*

Substrate	μmole of O_2 per μmole of substrate	
	Experimental	Theoretical[a]
DL-Methionine	4.6	3.5
α-Keto butyrate	2.8	2.5
Propionate	1.8	1.5
Succinate	1.5	1.5
α-Amino butyrate	3.8	3.5

[a] Assuming that oxidation beyond succinate was the same as for succinate, and that for methionine there was oxidative deamination and oxidative decarboxylation of the resulting keto butyrate (reactions 1 and 4 in text).

TABLE 3. *Effect of malonate on the oxidation of postulated intermediates by washed pregrown cells of A. starkeyi*

Substrate[a]	$Q(O_2)$[b]		$^{14}CO_2$ liberated[c]	
	No inhibitor	Malonate, 5 mM	No inhibitor	Malonate, 5 mM
DL-Methionine	14.8	0		
α-Keto butyrate	3.8	0		
Propionate-*1*-^{14}C	2.3	0	250,000	1,299
Succinate	6.6	0		
α-Amino butyrate-*1*-^{14}C	4.8	0	172,400	25,624

[a] Two micromoles.

[b] Expressed as microliters of O_2 per minute per milligram of protein. Values were calculated from the slopes of the oxidation curves.

[c] Specific activity of the propionate was 0.5 μCi per μmole, and of the α-amino butyrate, 0.25 μCi per μmole. Values expressed as counts per minute.

extracted with ether in a liquid-liquid continuous extractor after acidification with concentrated H_2SO_4. The extracted acids were analyzed by paper chromatography with isoamyl alcohol saturated with 4 M formic acid (12). The results showed that succinic acid had accumulated.

Tests on cell-free extracts of the bacterium were made for the following enzymes which were presumed to be involved in dissimilation of methionine: amino acid oxidase, α-keto butyrate oxidase, propionic acid kinase, propionyl-CoA carboxylase, methyl malonyl CoA mutase, and succinic acid dehydrogenase. Tables 4 to 8 and Fig. 3 show that all of these enzymes were present, but the level of propionyl-CoA carboxylase was low.

TABLE 4. *Amino acid oxidase in cell-free extract of A. starkeyi*

Conditions	Ketoacid	μmoles of ketoacid per min per mg of protein	
		Total	Net[a]
	μmoles		
Complete system....	2.44	40 × 10³	28 × 10³
No methionine......	0.77	12 × 10³	0
No enzyme..........	0	0	0

[a] Without methionine.

TABLE 5. *Decarboxylation of α-amino butyrate by cell-free extract of A. starkeyi*

Conditions	$^{14}CO_2$	CO_2 per min per mg of protein[a]	
		Total	Net[b]
Complete system........	5,350	5,842 × 10³	5,522 × 10³
No amino acid oxidase or catalase............	7,854	8,700 × 10³	8,380 × 10³
No lipoic acid or DPT[c]..	5,788	6,321 × 10³	6,001 × 10³
No enzyme.............	293	320 × 10³	0

[a] Expressed as counts per minute.
[b] Without enzyme.
[c] Diphosphothiamine.

TABLE 6. *Propionate kinase in cell-free extract of A. starkeyi*

Conditions	Hydroxamate formed	μmoles of hydroxamate per min per mg of protein	
		Total	Net[a]
	μmoles		
Complete system..	0.440	61 × 10³	50.5 × 10³
No CoA-SH......	0	0	0
No propionate....	0.018	2.5 × 10³	0
No enzyme.......	0.055	8 × 10³	0

[a] Without propionate or enzyme.

TABLE 7. *Propionyl CoA carboxylase in cell-free extract of A. starkeyi*

Conditions	Ether-soluble compounds[a]	$^{14}CO_2$ incorporated per min per mg of protein[a]
Complete system.......	540	9,000 × 10³
No propionyl CoA.....	1	16 × 10³ M
Propionate and CoA-SH instead of propionyl CoA..........	20	320 × 10³
No enzyme.............	1	16 × 10³

[a] Expressed as counts per minute.

TABLE 8. *Occurrence of methyl malonyl-CoA mutase in cell-free extract of A. starkeyi*

Conditions	Absorbancy[a]	μmoles of DCPIP[b] oxidized per min per mg of protein	
		Total	Net[c]
Complete system....	0.305	4.0 × 10³	2.9 × 10³
No methyl malonyl CoA.............	0.100	1.1 × 10³	0
No enzyme..........	0	0	

[a] Values expressed as change in absorbancy per minute.
[b] 2,6-Dichlorophenol indophenol.
[c] Without methyl malonyl CoA.

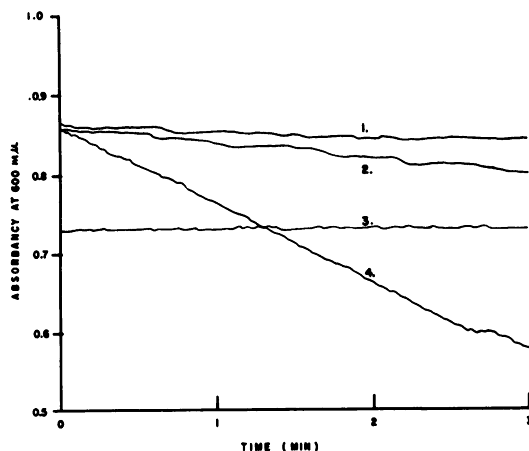


FIG. 3. *Determination of succinic dehydrogenase in cell-free extract of A. starkeyi. Lines are tracings from the recorder. Line 1, no substrate; line 2, no phenazine methosulfate; line 3, no enzyme; line 4, complete system. A change of 0.1 in absorbancy corresponds to oxidation of 0.0157 nmoles of succinate.*

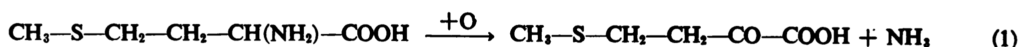
DISCUSSION

Our results show that the first step in the degradation of methionine by *A. starkeyi* is its oxidative deamination to α-keto methionine which is the substrate for demethiolation. The following evidence supports this hypothesis. (i) Semicarbazide, iodoacetate, and PCMB inhibited demethiolation but not deamination and, in the presence of semicarbazide, the only keto acid recovered was the semicarbazone of α-keto methionine; (ii) α-keto methionine accumulated during bacterial growth and disappeared subsequently; (iii) decomposition of α-keto methionine by washed cells was more rapid than that of methionine.

The fact that oxygen was required for demethiolation of both methionine and α-keto methionine suggests that oxygen is required for permeation of α-keto methionine or that cleavage of the C-S bond with release of methanethiol involves an oxidative step. The probability that α-keto methionine and not methionine is the substrate for demethiolation was suggested by Challenger and Charlton (8), Canellakis and Tarver (6; E. S. Canellakis and H. Tarver, Fed. Proc., p. 194, 1952), Miwatani et al. (24), and Segal and Starkey (30) based on the observed rates of deamination and demethiolation. Different results were obtained by Wiesendanger and Nisman (35) with *Clostridium* sp., by Kallio and Larson (15) with *Pseudomonas* sp., and by us with *Aspergillus* sp. (29), from which it was concluded that both deamination and demethiolation were carried out by the same enzyme.

With respect to the sulfur products, our results are similar to those obtained by Segal and Starkey (30) with a bacterial culture and by us with *Aspergillus* sp. (27, 28). Both methanethiol and dimethyl disulfide were formed but, based on the times of their appearance, it is concluded that the disulfide is formed by oxidation of the thiol.

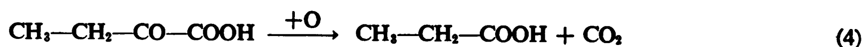
Our data suggest that *A. starkeyi* dissimilates methionine by the following series of reactions.



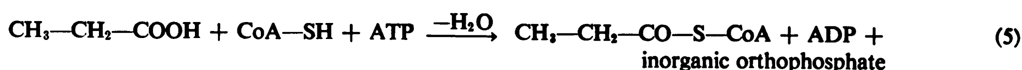
$$CH_3{-}S{-}CH_2{-}CH_2{-}CH(NH_2){-}COOH \xrightarrow{+O} CH_3{-}S{-}CH_2{-}CH_2{-}CO{-}COOH + NH_3 \quad (1)$$

$$CH_3{-}S{-}CH_2{-}CH_2{-}CO{-}COOH \xrightarrow{+2H} CH_3{-}CH_2{-}CO{-}COOH + CH_3{-}SH \quad (2)$$

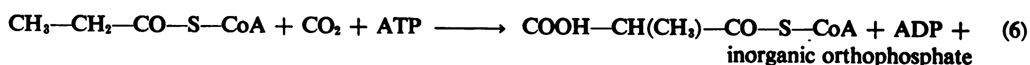
$$2CH_3{-}SH \xrightarrow{-2H} H_3C{-}S{-}S{-}CH_3 \quad (3)$$



$$CH_3{-}CH_2{-}CO{-}COOH \xrightarrow{+O} CH_3{-}CH_2{-}COOH + CO_2 \quad (4)$$



$$CH_3{-}CH_2{-}COOH + CoA{-}SH + ATP \xrightarrow{-H_2O} CH_3{-}CH_2{-}CO{-}S{-}CoA + ADP + \text{inorganic orthophosphate} \quad (5)$$



$$CH_3{-}CH_2{-}CO{-}S{-}CoA + CO_2 + ATP \longrightarrow COOH{-}CH(CH_3){-}CO{-}S{-}CoA + ADP + \text{inorganic orthophosphate} \quad (6)$$

$$COOH{-}CH(CH_3){-}CO{-}S{-}CoA \longrightarrow COOH{-}CH_2{-}CH_2{-}CO{-}S{-}CoA \quad (7)$$

$$COOH{-}CH_2{-}CH_2{-}CO{-}S{-}CoA \xrightarrow{+H_2O} COOH{-}CH_2{-}CH_2{-}COOH + CoA{-}SH \quad (8)$$

$$COOH{-}CH_2{-}CH_2{-}COOH \longrightarrow \text{To trichloroacetic acid cycle} \quad (9)$$

The concept that α-keto butyric acid, propionic acid, methyl malonate, and succinic acid are intermediates in degradation of methionine is supported by the following evidence. (i) Induced cells oxidized all of the postulated intermediates except methyl malonate, and oxidation of these compounds by noninduced cells was inhibited by CAP; (ii) propionic acid, α-keto butyric acid, and succinic acid accumulated when the cells were incubated with methionine; (iii) malonic acid inhibited oxidation of the postulated intermediates; (iv) the enzymes required to bring about the series of reactions were detected in induced cells with one important exception. The enzyme which was not detected in the cell extract is the demethiolase that releases methanethiol from α-keto methionine. Many attempts to obtain this enzyme in cell-free extracts failed, but additional attempts are being made to isolate the enzyme and establish its mode of action.

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