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Metabolism of 5-Methylthioribose to Methionine[1]

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ABSTRACT

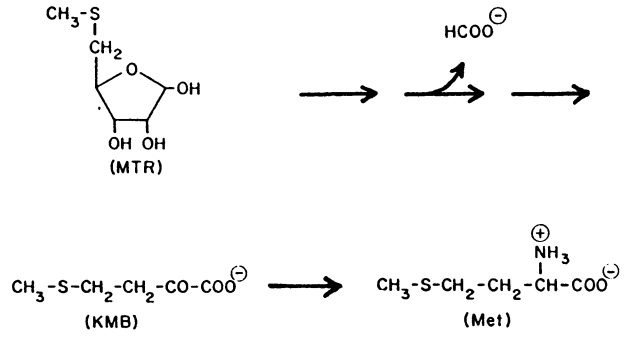
During ethylene biosynthesis, the H_3CS- group of *S*-adenosylmethionine is released as 5′-methylthioadenosine, which is recycled to methionine via 5-methylthioribose (MTR). In mungbean hypocotyls and cell-free extracts of avocado, [^{14}C]MTR was converted into labeled methionine via 2-keto-4-methylthiobutyric acid (KMB) and 2-hydroxy-4-methylthiobutyric acid (HMB), as intermediates. Incubation of [ribose-U-^{14}C]MTR with avocado extract resulted in the production of [^{14}C]formate, indicating the conversion of MTR to KMB involves a loss of formate, presumably from C-1 of MTR. Tracer studies showed that KMB was converted readily *in vivo* and *in vitro* to methionine, while HMB was converted much more slowly. The conversion of KMB to methionine by dialyzed avocado extract requires an amino donor. Among several potential donors examined, L-glutamine was the most efficient. Anaerobiosis inhibited only partially the oxidation of MTR to formate, KMB/HMB, and methionine by avocado extract. The role of O_2 in the conversion of MTR to methionine is discussed.

The amino acid methionine is a precursor of ethylene in higher plants. The biosynthetic pathway has been established as follows: Methionine → SAM[2] → ACC → ethylene (2). Since the methionine concentration in apple tissue is too low to sustain continued ethylene production, Baur and Yang (5) suggested that the methionine sulfur atom must be recycled to replenish the methionine pool. Adams and Yang (1, 2) subsequently showed that the methylthio (H_3CS-) moiety of SAM was released as MTA with the concomitant production of ACC. The MTA underwent hydrolytic cleavage to yield MTR, from which the methylthio group was recycled to form methionine. It was first assumed that MTR donated the methylthio group to an acceptor molecule, such as homoserine, for methionine regeneration (1). However, subsequent studies revealed that MTR provides both the methylthio and 2-aminobutyrate portions of methionine in plants (14, 15), as it does in animals (4) and bacteria (11).

In cell-free avocado extracts, Kushad *et al.* (9) recently have demonstrated that MTR is converted to KMB and HMB in the presence of ATP, whereas the conversion of MTR-1-P to these products is ATP-independent. These *in vitro* results indicate that MTR is first phosphorylated to MTR-1-P, which is then metabolized to KMB. KMB presumably is transaminated to form methionine. Little is known about the conversion of MTR-1-P to KMB. As the five-carbon ribose moiety of MTR is transformed into the four-carbon 2-ketobutyrate portion of KMB, one of the five carbons of the ribose moiety of MTR must be released during the conversion. In rat liver extracts, Trackman and Abeles (12) observed that MTR is converted into KMB with the stoichiometric consumption of O_2 and production of HCOOH, indicating that C-1 of MTR-1-P was released as formate. In this study, we present *in vivo* data showing that MTR is indeed converted into methionine via KMB as an intermediate, and that the conversion of MTR to KMB involves the stoichiometric release of formic acid (Scheme 1).

MATERIALS AND METHODS

Chemicals. L-[Methyl-^{14}C]methionine and [methyl-^{14}C]SAM were purchased from Research Products International, Mount Prospect, IL. [Ribose-U-^{14}C]MTA was kindly provided by Dr. Fritz Schlenk, University of Illinois, Chicago, IL. KMB sodium salt, catalase, and L-amino acid oxidase were obtained from Sigma Chemical Co. HMB calcium salt came from Nutritional Biochemicals Corp., Cleveland, OH. [Methyl-^{14}C]MTR was prepared from [methyl-^{14}C]SAM as described previously (15); methionine was added to the final product at a concentration of 0.5 mM to prevent oxidation of the MTR to its sulfoxide. [Ribose-U-^{14}C]MTR was prepared from [ribose-U-^{14}C]MTA as previously described (15). For the preparation of [methyl-^{14}C]KMB, L-[methyl-^{14}C]methionine was incubated with L-amino acid oxidase and catalase overnight at 25°C. An equal volume of 95% (v/v) ethanol was added and the solution centrifuged to remove any precipitate. The supernatant was evaporated under N_2, redissolved in water, and passed through the cation exchange resin Dowex 50 (H^+ form) to remove residual methionine. The effluent containing the KMB was concentrated. [Methyl-^{14}C]HMB was prepared by adding a small amount of $NaBH_4$ to the [methyl-^{14}C]KMB prepared above. Excess borohydride was destroyed by the addition of HCl, and the solution then neutralized with NaOH. Purity of all radiochemicals prepared was verified by paper chromatography on Whatman No. 1 paper developed in *n*-butanol:acetic acid:water (4:1:1, v/v/v) and by paper electrophoresis.



SCHEME 1

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[2] Abbreviations: SAM, *S*-adenosylmethionine; ACC, 1-aminocyclopropane-1-carboxylic acid; MTA, 5′-methylthioadenosine; MTR, 5-methylthioribose; KMB, 2-keto-4-methylthiobutyric acid; HMB, 2-hydroxy-4-methylthiobutyric acid; MTR-1-P, 5-methylthioribose-1-phosphate; AOA, aminooxyacetic acid.

Feeding Experiment. Mungbean (*Vigna radiata* cv Berken) seedlings were grown in the dark for 3 d at 25°C. Two cm-long hypocotyl segments were cut below the hook, immediately rinsed in 50 mM K-phosphate (pH 7.0), and blotted dry on paper towels. Mungbean segments weighing about 0.75 g were placed upright in a 5 ml glass vial with 0.15 ml of feeding solution, which contained 50 mM K-phosphate (pH 7.0), plus the specified radioactive and nonradioactive compounds. For incubation under anaerobic conditions, the vial containing the hypocotyls was placed in a syringe and flushed with N_2. The syringe was then sealed and placed under water in a large graduated cylinder to prevent diffusion of air into the syringe. All samples were incubated at 25°C for 5 h. After incubation, the mungbean segments were rinsed in 50 mM K-phosphate (pH 7.0), homogenized, and extracted in 80% (v/v) ethanol overnight. The extracts were concentrated *in vacuo*. Radiolabeled metabolites were analyzed by paper chromatography on Whatman No. 1 paper using 1-butanol:acetic acid:water (4:1:1, v/v/v) as the developing solvent, and by paper electrophoresis at pH 1.9 (HCOOH:acetic acid:water, 2.5:7.5:90 v/v/v), pH 4.0 (0.2 M acetate buffer), or at pH 7.0 (20 mM K-phosphate). Radioactivity was detected by a Packard radiochromatogram scanner. Methionine, KMB, HMB, and MTR standards were visualized by iodoplatinate reagent (3). Extracts also were fractionated into cationic, anionic, and neutral fraction by passage in series through a cation exchange resin (Dowex 50-H^+) and an anion exchange resin (Dowex 1-formate). Compounds which did not bind to either resin were taken to be neutral. Anions and cations were eluted from their resins with 6 N HCOOH and 2 N NH_4OH, respectively. Fractions were concentrated, and the radioactivity in each fraction was quantitated by scintillation counting. Metabolites in each fraction were separated by paper chromatography and paper electrophoresis as described above.

Avocado Cell-free Extract. Avocado (*Persea americana* cv Hass) cell-free extract was prepared by homogenizing tissue in buffer containing 50 mM K-phosphate (pH 7.0). 3 mM DTT, and 0.3% (w/v) PVP (9). For each gram of tissue, 1 ml of buffer was used. The homogenate was centrifuged at 20,000*g* for 20 min, and the supernatant was used as a crude enzyme preparation. If an extract lacking cofactors was desired, the supernatant was dialyzed overnight against buffer containing 50 mM K-phosphate (pH 7.0) and 0.5 mM DTT. All steps of this preparation were carried out at 4°C. A typical reaction mixture contained 50 to 500 μl avocado extract, 3 mM DTT, 1 mM L-glutamine, 1 mM ATP, 5 mM $MgSO_4$, and [^{14}C]MTR, in a total volume of 0.1 to 1 ml. When labeled KMB was employed, the reaction mixture was similar to that above, except that ATP and MTR were omitted. The reaction mixtures were incubated at 25°C for 1 to 5 h. At the end of the incubation, the reaction mixtures were boiled for 5 min, centrifuged to remove the denatured protein, and analyzed for reaction products as described above. Labeled formate was analyzed as described below.

Formaldehyde/Formate Assays. Radioactive formaldehyde and HCOOH, which were formed from [ribose-U-^{14}C]MTR in the above reaction mixtures with avocado extracts, were determined by enzymic oxidations of formaldehyde to CO_2 with both formaldehyde dehydrogenase and formate dehydrogenase, and of formate to CO_2 with formate dehydrogenase. Approximately 0.2 ml of the reaction mixtures was placed in a 25 ml Erlenmeyer flask, to which was added 0.3 ml of 50 mM K-phosphate (pH 7.0), containing 0.2 unit of formaldehyde dehydrogenase and/or 0.2 unit of formate dehydrogenase. The reaction was initiated upon the introduction of 1 μmol of NAD^+ to the assay mixture. The flask was sealed with a serum cap, and the radioactive CO_2 released during the oxidation was absorbed into a plastic well containing a strip of paper wetted with KOH solution. After incubation for 1 h at room temperature, 1.0 ml of 1 M K-phosphate (pH 3), was injected into the flask to release any remaining CO_2 from the solution. The amount of formate was taken to be the amount of $^{14}CO_2$ obtained following the formate dehydrogenase oxidation, whereas the amount of formaldehyde was taken to be the amount of $^{14}CO_2$ obtained from the combined formaldehyde dehydrogenase and formate dehydrogenase oxidations minus that from the formate dehydrogenase oxidation. $^{14}CO_2$ was quantitated by counting the radioactivity in the plastic well by liquid scintillation.

RESULTS AND DISCUSSION

KMB and HMB as Intermediates in the Metabolism of MTR to Methionine *in Vivo*. When mungbean hypocotyls were administered [methyl-^{14}C]MTR, the major radioactive product was identified as methionine, based on the observations that this metabolite was adsorbed by the cation exchange resin Dowex 50 (H^+) and co-migrated with authentic methionine in paper chromatography (R_F = 0.41) and paper electrophoresis at pH 1.9 (Fig. 1). Thus, MTR is converted to methionine *in vivo*. When 5 mM unlabeled KMB was added to the feeding solution, two new compounds with R_F of 0.70 and 0.80 on the paper chromatograms were observed. When the mungbean extract was fractionated into cationic, anionic, and neutral fractions, both compounds were found in the anionic fraction. The compound with R_F = 0.70 co-migrated with authentic KMB in both paper chromatography and electrophoresis at pH 4, while that with R_F = 0.80 co-migrated with HMB. Analysis of the distribution of radioactivity in the cationic, anionic, and neutral fractions revealed that in the sample fed unlabeled KMB, as compared to that without KMB, the radioactivity in the cationic fraction (consisting largely of methionine) decreased from 66 to 40% of the total radioactivity, while that of the anionic fraction (consisting mainly of KMB and HMB) increased from 16 to 40%. Similar trends were observed when unlabeled HMB was administered replacing KMB. These data suggest that both KMB and HMB are produced from MTR during its conversion to methionine *in vivo*, but are readily converted into methionine and consequently do not accumulate in significant amounts; administration of unlabeled KMB or HMB, however, reduces the

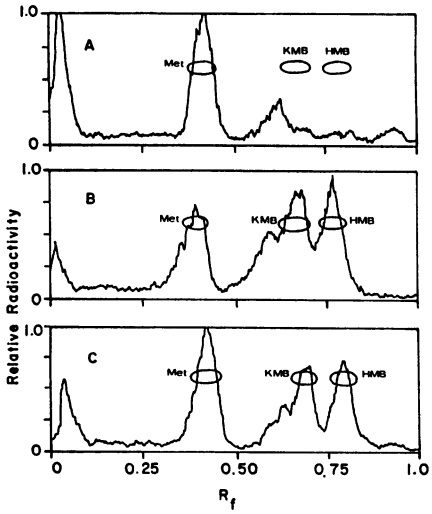

FIG. 1. Metabolism of [^{14}C]MTR in mungbean hypocotyls. Paper radioachromatogram scans of ethanol extracts of mungbean segments administered [methyl-^{14}C]MTR (A), [methyl-^{14}C]MTR + 0.75 μmol KMB (B), or [methyl-^{14}C]MTR + 0.75 μmol HMB (C). The amount of [methyl-^{14}C]MTR administered was 0.15 μCi (38 μCi/umol), and the samples were incubated for 5 h. Spots represent the positions of authentic compounds.

conversion of [^{14}C]KMB/HMB to [^{14}C]methionine due to isotope dilution. Indeed, when [methyl-^{14}C]KMB or [methyl-^{14}C]HMB was administered to mungbean segments, both KMB and HMB were found to be converted to methionine as shown in Figure 2. However, the percentage of HMB converted to methionine (17%) was lower than that of KMB (72%). It should be noted, however, that in this study [^{14}C]HMB was synthesized by reduction of [^{14}C]KMB with borohydride, which possibly yielded a racemic mixture of HMB. If the enzymes that convert HMB to methionine are stereospecific, only half of the HMB can be metabolized. Thus, the percentage of conversion of HMB may be underestimated.

Since HMB is converted to methionine, it is pertinent to ask whether the HMB-to-methionine conversion proceeds via KMB as an intermediate. Langer (10) proposed that in rat liver, HMB was first oxidized to KMB, which was then transaminated to form methionine. If HMB is metabolized to methionine via KMB, then compounds that inhibit the conversion of KMB to methionine should also inhibit the conversion of HMB to methionine. Since it is likely that KMB is converted to methionine by a transaminase, we studied the effect of AOA, a transaminase inhibitor, on the conversion of KMB and HMB to methionine. However, this approach was unsuccessful, because the AOA formed an adduct with the KMB, as determined by paper chromatography. In addition, we compared the conversion of [^{14}C]HMB into methionine in the presence or absence of excess unlabeled KMB. If HMB is converted to methionine via KMB, unlabeled KMB should inhibit the conversion of [^{14}C]HMB to [^{14}C]methionine. Although the unlabeled KMB did cause a decrease in the conversion of [^{14}C]HMB to methionine (from 17 to 13%), the decrease was too small to be significant.

Conversion of MTR to Methionine *in Vitro*. Kushad *et al.* (9) have reported that crude avocado cell-free extract converts MTR largely to KMB and HMB in the presence of ATP, and that the extract is capable of converting the KMB, but not HMB, to methionine. However, we observed that [^{14}C]MTR was metabolized by crude avocado extract largely to [^{14}C]methionine and [^{14}C]HMB (Fig. 3), and that the extract could convert both KMB and HMB to methionine, although the conversion of HMB to methionine proceeded at a much lower rate than that of KMB to methionine. Addition of 5 mM unlabeled HMB to the reaction mixture containing [^{14}C]MTR did not significantly affect the metabolite profile over that without HMB. When 5 mM unlabeled KMB was added, however, both [^{14}C]KMB and [^{14}C]HMB accumulated, with little [^{14}C]methionine produced. Thus, the *in vitro* avocado extract system differs from the *in vivo* mungbean hypocotyl system in that addition of excess unlabeled KMB or HMB caused accumulation of [^{14}C]KMB from [^{14}C]MTR in

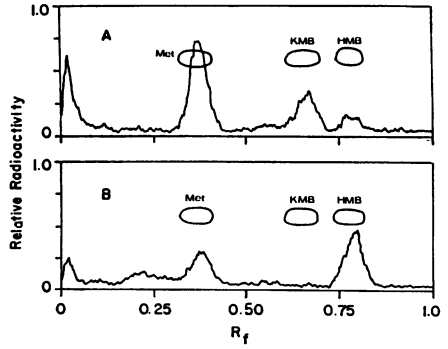

FIG. 2. Conversion of KMB and HMB to methionine by mungbean hypocotyl segments. Paper radiochromatogram scans of ethanol extracts of mungbean segments administered [methyl-^{14}C]KMB (A), or [methyl-^{14}C]HMB (B), and incubated for 5 h. The amount of labeled KMB or HMB administered was 0.13 μCi (1.3 μCi/umol).

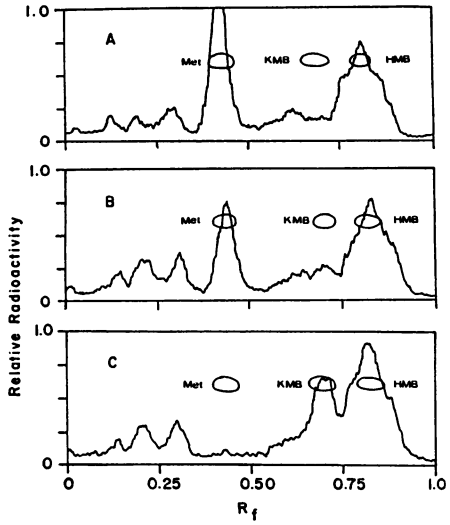

FIG. 3. Metabolism of MTR in avocado cell-free extract. Paper radiochromatogram scans of avocado extract incubated with [methyl-^{14}C]MTR (A), [methyl-^{14}C]MTR + 0.5 μmol HMB (B), or [methyl-^{14}C]MTR + 0.5 μmol KMB (C). The amount of [methyl-^{14}C]MTR used in each sample was 0.11 μCi (47 μCi/μmol). Each reaction mixture contained 0.3 μmol DTT, 5 μmol K-phosphate (pH 7), 0.5 μmol $MgSO_4$, 0.1 μmol ATP, and 50 μl avocado extract, in a total volume of 0.1 ml, and was incubated 5 h.

mungbean hypocotyls, whereas only KMB, but not HMB, caused this accumulation in avocado extract. These data indicate that both HMB and KMB are metabolites of MTR, and that KMB and HMB are interconvertible in mungbean hypocotyls, but HMB is not readily converted to KMB in avocado extract. It is possible that a cofactor is required for the conversion of HMB to KMB, but the level of this cofactor is too low in the avocado extract to effect the conversion.

A question thus arises as to the metabolic role of HMB. Since KMB is more efficiently converted to methionine than HMB, KMB appears to be closer than HMB to the immediate precursor of methionine in the MTR-to-methionine pathway. One possibility is that MTR is metabolized to KMB via HMB as an intermediate, namely MTR → HMB → KMB → Methionine. However, such a sequence is not compatible with our data obtained with avocado extract, which readily converted MTR to KMB, but had a rather limited capability to convert HMB to KMB. From these observations, coupled with our *in vivo* results that HMB is formed from MTR, that HMB is converted to methionine, and that KMB and HMB are interconvertible, we suggest that HMB is a side-product of the conversion of MTR to methionine, as shown below:

$$\text{MTR} \rightarrow \text{MTR-1-P} \longrightarrow \text{KMB} \rightarrow \text{Methionine}$$
$$\Downarrow\Uparrow$$
$$\text{HMB}$$

We have attempted to fractionate the enzymes in avocado extract which catalyze the conversion of MTR to KMB, using such methods as $(NH_4)_2SO_4$, fractionation, DEAE cellulose chromatography, and gel filtration chromatography. However, these attempts were unsuccessful.

We have observed that crude avocado extract is capable of converting KMB to methionine without the addition of any cofactors. When the avocado extract is first dialyzed against 50 mM K-phosphate, however, KMB is not converted to methionine without the addition of a suitable amino group donor. Various amino acids, at concentrations of 1 mM, were tested for their abilities to effect the conversion of KMB to methionine. Among

the amino donors tested, L-glutamine was the most effective, with which 68% of the KMB was converted to methionine under the assay conditions used. L-Glutamate was about one-third as effective as L-glutamine, while D- and L-asparagine, D- and L-aspartate, and D-glutamate were only slightly effective (Table I). Presumably the α-amino group of glutamine was transferred to KMB in a transamination reaction. Although our data do not indicate whether the nitrogen transferred was the α-amino or amide nitrogen of glutamine, Ireland (8) has shown that glutamine can donate its α-amino group to KMB to form methionine in a reaction catalyzed by pea leaf glutamine aminotransferase. In rat liver, both glutamine and asparagine were found to donate their amino groups to KMB (6, 7). In contrast to the animal system, however, asparagine is a poor donor of amino groups to KMB in avocado extract (Table I). In their assays of the KMB-to-methionine conversion in avocado extract, Kushad *et al.* (9) added 1 mM asparagine, presumably to serve as an amino donor and facilitate the conversion. Our results, however, indicate that L-glutamine is a much better amino donor. We were not able to detemine whether L-glutamine also serves as the amino donor *in vivo*.

Release of Formate during the Conversion of MTR to KMB. In the conversion of the six-carbon MTR into the five-carbon KMB, one of the carbons, presumably carbon-1 of MTR's ribose moiety, must be released. To determine the form in which this carbon is released, we fed [ribose-U-^{14}C]MTR to crude cell-free avocado extracts in the presence of ATP, and assayed for labeled one-carbon compounds—CO_2, formate, and formaldehyde—in relation to methionine formation. Although some $^{14}CO_2$ was released during the incubation, this amount was small when compared to that of formate (Table II). No formaldehyde wa detected, however. Little CO_2 and formate were released when the extract was boiled for 5 min before the incubation, indicating that the CO_2 and formate were formed enzymically. It is possible that the actual form of the released carbon is formaldehyde, which subsequently is oxidized to formate. However, attempts to trap [^{14}C]formaldehyde by incubating the reaction mixtures in the presence of up to 1 mM unlabeled formaldehyde did not result in any accumulation, indicating that [^{14}C]formaldehyde was not present.

Table I. *Effect of Various Amino Acids on the Conversion of KMB to Methionine by Dialyzed Avocado Extract*

The reaction mixtures contained 0.3 μmol DTT, 5 μmol K-phosphate (pH 7), 50 μl dialyzed avocado extract, 11 nCi [methyl-^{14}C]KMB (1 nCi/nmol) and 0.1 μmol of the specified amino acid, in a total volume of 0.1 ml. After incubation for 4 h, the radioactive methionine formed was determined and expressed as percent of total radioactivity.

Amino Acid	Methionine
	%
None	0
D-Ala	11
L-Ala	8
D-Asn	7
L-Asn	7
D-Asp	0
L-Asp	1
D-Gln	7
L-Gln	68
D-Glu	2
L-Glu	19
Gly	0
D-Met	21
L-Met	60
D-Ser	3
L-Ser	0

Table II. *Relationship between CO_2, Formic Acid, and Formaldehyde Release and Methionine Production from [ribose-U-^{14}C]MTR in Avocado Extract*

The reaction mixture contained 0.15 μmol ATP, 0.45 μmol DTT, 0.75 μmol $MgSO_4$, 11 nCi [ribose-U-^{14}C]MTR (0.75 nCi/nmol), and 75 μl of avocado extract in a total volume of 0.15 ml. After incubation for 5 h, the radioactivity in the specified compounds was determined. The amount of each product was calculated from its radioactivity, assuming that the specific radioactivities of CO_2, HCOOH, and formaldehyde were 0.15 nCi/nmol (one-fifth of the specific radioactivity of the substrate), and that of methionine was 0.60 (four-fifths of the specific radioactivity of the substrate).

CO_2	HCOOH	Formaldehyde	Methionine
	nmol		
0.6	3.4	0	3.1

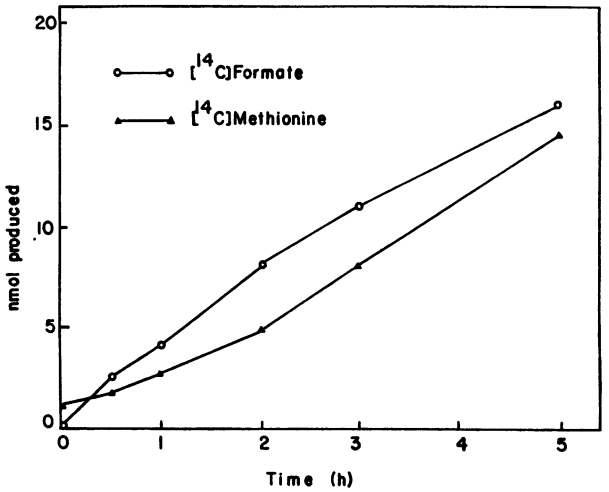

FIG. 4. Time course of labeled methionine and formate production from [ribose-U-^{14}C]MTR by avocado extract. The reaction mixture contained 3 μmol ATP, 9 μmol $MgSO_4$, 15 μmol DTT, 0.15 mmol K-phosphate (pH 7), 0.11 μCi [ribose-U-^{14}C]MTR (0.75 μCi/μmol), and 1.5 ml avocado extract, in a total volume of 3.0 ml. The sample was incubated at 25°C, and aliquots (0.5 ml) were periodically withdrawn for the determination of radioactive formate and methionine.

If formate is a product in the conversion of MTR to methionine, then there should be a stoichiometric relation between the amounts of formate and methionine produced over time. Figure 4 shows such a relationship. The production of labeled methionine paralleled that of labeled formate. The amount of formate produced on a molar basis at any given time was slightly more that of methionine. Part of the difference can be attributed to the presence of KMB and HMB, which are known to be present in the reaction mixture but are not accounted for in this calculation.

Oxygen Requirement for the Conversion of MTR to Methionine. The conversion of MTR to KMB and HCOOH involves a four-electron oxidation. Recently, Trackman and Abeles (13) reported that in rat liver homogenates, MTR-1-P is first isomerized to methylthioribulose-1-phosphate. In the absence of O_2, two unidentified compounds were produced from methylthioribulose-1-P, but upon reexposure to O_2 these two compounds were converted to KMB with the uptake of O_2. In a separate study, Trackman and Abeles (12) found that MTR-1-P was converted into KMB with the stoichiometric consumption of O_2 and production of HCOOH. Wang *et al.* (14) have determined that the conversion of MTR to methionine in tomato fruit tissue is highly O_2 dependent. To define further the biochemical role of O_2 in the pathway, we examined the effect of anaerobiosis on

the conversion of [methyl-^{14}C]MTR to methionine by mungbean segments in the presence of unlabeled KMB. The unlabeled KMB was added to cause accumulation of both labeled KMB and methionine, so that we could determine whether anaerobiosis affects KMB production, as well as methionine production. Metabolites were extracted and separated into cationic, anionic, and neutral fractions. The anaerobic environment did not greatly affect the uptake of [^{14}C]MTR by the mungbean segments but did inhibit its metabolism to methionine, compared to the samples incubated aerobically. In contrast to the results of Wang *et al.* (14), who reported complete inhibition of the metabolism of MTR to methionine by anaerobiosis, we were only able to achieve partial inhibition (55%) with mungbean hypocotyls. This inhibition is reflected in the decreased radioactivity in the cationic (mainly methionine) and anionic (mainly KMB and HMB) fractions, compared to the aerobic control sample. In the aerobic sample, the percentages of radioactivity fed in as [^{14}C]MTR and recovered in the cationic and anionic fractions were 33 and 44%, respectively. The corresponding values in the anaerobic sample were 12 and 21%, respectively. These results indicate that O_2 enhances the conversion of MTR to methione *in vivo*, and that the O_2-requiring step lies between MTR and KMB in the pathway, since anaerobiosis inhibited the formation of KMB, as well as methionine. In the anaerobic samples administered [^{14}C] MTR, most of the radioactivity was recovered in the neutral fraction (67%). About half of this radioactivity was due to unmetabolized [^{14}C]MTR, and the other half was due to an unidentified compound with $R_F = 0.75$ on the paper chromatogram. This compound was not present in the aerobic sample. When this compound was isolated and refed to mungbean segments under aerobic conditions, it was not metabolized. Thus, it appears that this unknown compound, which was produced from MTR under anaerobic conditions, was not an intermediate in, and thus unrelated to, the MTR-to-methionine pathway.

To investigate further the O_2 requirement, we examined the effect of anaerobiosis on methionine, KMB/HMB, and formate production from [ribose-U-^{14}C]MTR in the presence of ATP by crude avocado extract. Anaerobic conditions in the reaction mixtures were achieved by employing glucose and glucose oxidase and bubbling N_2. Assay of the O_2 concentration by the Clark O_2 electrode indicated that the O_2 concentration was maintained below 2 μM. Under such anaerobic conditions, the production of [^{14}C]formate and the fraction of radioactivity present as [^{14}C]KMB/HMB/methionine decreased approximately 30 to 40% in the anaerobic sample compared to the aerobic sample. These results are in agreement with the data of the mungbean hypocotyls that anaerobiosis inhibited only partially the conversion of MTR to KMB and methionine.

Our observation that anaerobiosis did not totally inhibit the conversion of MTR to methionine *in vivo* and *in vitro* may be explained as follows: (1) an enzyme in this pathway requires O_2 as the electron acceptor, but its affinity for O_2 is so high that residual amounts of O_2 remaining in the tissue or reaction mixture after anaerobic treatment are sufficient to support KMB production, or (b) the system utilizes a redox carrier as the immediate electron acceptor and molecular O_2 as the ultimate electron acceptor; therefore, anaerobiosis would not totally inhibit the MTR-to-KMB oxidation, and the extent of the inhibition by anaerobiosis would depend on the concentration of the endogenous electron acceptor already present in the system.

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