Methionine as a Dominant Precursor of Phytosiderophores in Graminaceae Plants

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Nine \(^{14}\text{C}\)-labeled amino acids and \(^{14}\text{C}\)-acetic acid from root tips of Fe-deficient Graminaceae plants (Hordeum vulgare, Oryzae sativa and Avena sativa) were surveyed to determine the precursor amino acid of phytosiderophores. The dominant precursor was methionine, which was incorporated into avenic acid → deoxymugineic acid → mugineic acid → epihydroxymugineic acid and/or hydroxymugineic acid in this order. Methionine sulfone or methionine sulfoxide may be important intermediates in going from methionine to avenic acid.

Key words: Graminaceae plants — HPLC — [1-\(^{14}\text{C}\)]-methionine — Phytosiderophore — Radioanalyzer.

Since the discovery (Takagi 1972) and determination of the chemical structure of phytosiderophores (Takemoto et al. 1978), many investigators studying Fe-deficiency have been very interested in knowing the precursor amino acid for phytosiderophore synthesis. However, this is difficult to determine because not much is known about the location of phytosiderophore synthesis. Whether phytosiderophores are produced in the roots or the shoots is not clear. They have been found in the roots, xylem sap and shoots in Fe-deficient Graminaceae plants (Mori et al. 1987). Another reason for the lack of information is that when intact Fe-deficient plants are used, the data vary depending on the sterilization conditions of the roots, plant age, root volume, time of absorption, and the radioactive amount and the specific activity of radioactive amino acids. After many trials, we finally succeeded in establishing a system for phytosiderophore synthesis. This report explains the establishment of the survey system and the identification of the precursor of phytosiderophores using this system. We also discuss the precursor-product relationships among five siderophores found in the Graminaceae plants studied.

Materials and Methods


Plant culture conditions—Seeds were germinated on a paper-towel soaked with distilled water in a dish covered with an aluminium sheet at 20°C for 2 days. After germination, the seeds were transplanted on a saran net floating on tap water at pH 5.5 in a greenhouse. For barley, room temperature was maintained at 20°C/14 h-light and 15°C/10 h-dark. For the other plants, room temperature was maintained at 26°C/light and 20°C/dark. After about ten days, 15 bundles of

Abbreviations: MA, mugineic acid; deoxy-MA, deoxymugineic acid; hydroxy-MA, hydroxymugineic acid; epihydroxy-MA, epihydroxymugineic acid; sulfone, methionine sulfone; sulfoxide, methionine sulfoxide; OPA, orthophthalaldehyde; Brij-35, polyoxyethylene lauryl alcohol ether; Hypo, sodium hypochlorite.

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three plants, each were transplanted into 20 liter plastic boxes covered with plastic boards with 15 holes of 2 cm in diameter. The nutrient concentration in tap water was gradually increased starting from 1 strength to 3 strength at the shooting stage of the 5th leaf. At that time Fe-deficiency treatment was started using deionized-redistilled water.

*Zea mays*, *Avena sativa* and *Oryzae sativa* plants were cultured under light (29°C/14 h, 320 \( \mu E/m^2\cdot s \)) and dark (20°C/10 h) with 70% humidity. *Hordeum vulgare* plants were cultured under light (18°C/14 h, 320 \( \mu E/m^2\cdot s \)) and dark (14°C/10 h). After the start of Fe-deficiency treatment, the root washings were collected at 4-day intervals following *Collection of root washings*. When the amounts reached maximum and remained constant, the following root-tip experiments and \(^{14}\)CO\(_2\)-fixation experiments were conducted.

**Composition of the standard (1 strength) culture solution** — N: 32 ppm (NO\(_3\)-N: 24 ppm, NH\(_4\)-N: 8 ppm), P\(_2\)O\(_5\): 16 ppm, K\(_2\)O: 32 ppm, CaO: 32 ppm, MgO: 32 ppm, B: 0.4 ppm, Mo: 0.04 ppm, Zn: 0.04 ppm, and Cu: 0.02 ppm were supplied as NH\(_4\)NO\(_3\), KH\(_2\)PO\(_4\), KCl, Ca(NO\(_3\))\(_2\)-4H\(_2\)O, MgSO\(_4\)-7H\(_2\)O, H\(_3\)BO\(_3\), MnCl\(_2\)-4H\(_2\)O, NaMoO\(_4\)-2H\(_2\)O, ZnSO\(_4\)-7H\(_2\)O, and CuSO\(_4\)-5H\(_2\)O, respectively.

**Collection of root washings** — Root washings were collected by soaking all plant roots in distilled water (3 liters) in a plastic plate for 5 h after the light had been turned on. The cationic fraction was prepared following the method of Takagi (1976). Phytosiderophores were analyzed with HPLC.

**Identification of the location of phytosiderohore synthesis in a corn root** — First the amount of phytosiderophore in the corn root was analyzed by obtaining a sample under the dark condition 1 min before the light was turned on to avoid loss of phytosiderophores via secretion from the root when the light was turned on (Takagi et al. 1984). Ten lateral roots of the corn plant were cut at 1 cm from the root tip forward the basal parts. Segments of the same parts were combined and ground with a pestle and extracted with 80% ethanol, and the amounts of phytosiderophores in each part were analyzed with HPLC.

**Feeding of \(^{1}\)C-Met to root tips of Fe-deficient plants** — When plants showed severe chlorosis, the root tips were slightly twisted and fatty, and the amount of phytosiderophores secreted per day from the roots became maximum and reached a steady state level. At that time, the root tips (1-cm long) were harvested with a razor. The tips were sterilized in a 50-ml flask containing 20-ml culture solution (10-strength concentration) containing the following antibiotics: penicillin G (Meijiseika Ltd. Tokyo) 10\(^{4}\) units, streptomycin sulfate (Meijiseika Ltd. Tokyo) 1.2 mg, chloromycetin (Sankyo Yakuhin Ltd. Tokyo) 0.24 mg and sepharolysine (Torii Yakuhin Ltd. Tokyo) 0.3 mg. The flask was shaken in an incubator rotating at 60 rpm at 30°C for 10 min. Then the solution was discarded, and the root tips were washed four times each with 10 ml of the culture solution (10 strength). In case of *Oryzae sativa* L., four 10-ml test tubes were used. Ten root-tips were incubated in 2 ml of culture solution containing 10 strength of the nutrient concentration and 12.5 \( \mu \)Ci of \(^{1}\)C-Met (S.A. 56 mCi/mmol, Amersham). The tubes were shaken at 120 rpm for 15, 30, 60 and 120 min. Next, 8 ml of 95°C ethanol was poured into the reaction mixture, and the root tips were ground with a pestle and filtered through Toyo No. 5c filter paper. The extracts were evaporated in vacuo, diluted with 200 \( \mu l \) of dilution buffer (2 n Li citrate pH 2.2), filtered through Eikurodisk (0.45 \( \mu m \), Gelman Science, Japan-Ltd) and kept frozen (\(-30^\circ\)C) until HPLC-radioanalysis. The overall assay system is summarized in Table 2. In the case of *Hordeum vulgare* and *Avena sativa*, \(^{1}\)C-Met was fed for 30 min or 120 min. Other conditions for the extraction process were as given in the case of *Oryzae sativa*.

\[^{1}\]C-Asp (232 mCi/mmol, Amersham), \[^{1}\]C-Thr (224 mCi/mmol, Amersham), \[^{1}\]C-Ser (173 mCi/mmol, Amersham), \[^{1}\]C-Asn (232 mCi/mmol, NEN), \[^{1}\]C-Glu (293 mCi/mmol, NEN), \[^{1}\]C-Gly (50.1 mCi/mmol, NEN), \[^{1}\]C-Ala (174 mCi/mmol, Amersham), \[^{1}\]C-Val (280 mCi/mmol, Amersham) and \[^{2}\]C-sodium acetate
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Table 1  Conditions for phytosiderophore analysis with HPLC

<table>
<thead>
<tr>
<th>Column:</th>
<th>AA-pak Li-type (6.0 mm I.D. × 200 mm) purchased from JASCO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column temp:</td>
<td>40°C.</td>
</tr>
<tr>
<td>Buffer:</td>
<td>0.15 n Li+, pH 2.75 (0.05 mol Li-citrate+30 ml methanol+100 μl n-caprylic acid, pH adjustment by 60% HClO₄, total 1 liter).</td>
</tr>
<tr>
<td>Buffer flow:</td>
<td>0.5 ml/min.</td>
</tr>
<tr>
<td>Reagent:</td>
<td>Hypo solution, pH 10.5 (24.7 g H₂BO₃+0.1 ml 10% sodium hypochlorite solution, pH adjustment by 45% KOH, total 1 liter).</td>
</tr>
<tr>
<td>Reagent temp:</td>
<td>50°C.</td>
</tr>
<tr>
<td>Reagent flow:</td>
<td>0.5 ml/min.</td>
</tr>
<tr>
<td>Wave length:</td>
<td>345 nm for excitation and 455 nm for emission.</td>
</tr>
</tbody>
</table>

(55 mCi/mmol, NEN) were also fed for 30 min to the root tips of ‘Ehimehakada No. 1’ as in the [1-¹⁴C]-Met feeding experiments. However, no significant incorporation into any of the phytosiderophores was observed in such a short time absorption experiment.

**Feeding of ¹⁴CO₂ to Fe-deficient barley by photosynthesis—**Na₂¹⁴CO₃ (5 mCi, S.A: 7 mCi/mmol, ICN) was oxidized with 10 ml of 6% HClO₄ and the evolved ¹⁴CO₂ was circularly introduced into a box (50 cm × 35 cm × 35 cm) for photosynthesis in a phytotron. The box contained ten Fe-deficient barley plants, ‘Minorimugi’, had been transplanted in a 3-liter Wagner pot with Fe-absent 2-strength concentration of the nutrient solution. Photosynthesis was allowed to occur for 8 h under 18°C with the light intensity of 300 μE/m².s. After that, the plants were removed from the box and cultured under 18°C/14-h light and 14°C/10-h dark (Mori et al. 1987). To identify the primary assimilates in root tips, five root tips (1 cm long) were cut at the end of the photosynthesis period, and their 80% ethanol-soluble fractions were analyzed with an HPLC-radioanalyzer. Root tips were also sampled at the 2nd and the 4th day after the photosynthetic experiment, and the radioactivity of the ethanol-soluble fraction was analyzed as above. On the next day after the photosynthetic experiment, root washings were collected and a cationic fraction was prepared (Takagi 1976) and analyzed using the HPLC-radioanalyzer. The radioactive peak fractions of each phytosiderophore were collected, mixed with liquid scintillator (ACS-II, NEN)

Table 2  Assay system for the precursor of phytosiderphores

<table>
<thead>
<tr>
<th>Test tube</th>
<th>10 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture solution</td>
<td>2 ml (10 strength concentration)</td>
</tr>
<tr>
<td>Number of root-tips</td>
<td>10</td>
</tr>
<tr>
<td>Radioactive compounds</td>
<td>10 μCi (S.A. 50 μCi/mmol)</td>
</tr>
<tr>
<td>Absorption time</td>
<td>30 min</td>
</tr>
<tr>
<td>Incubation temperature</td>
<td>25°C</td>
</tr>
<tr>
<td>Shaking condition</td>
<td>120 rpm</td>
</tr>
</tbody>
</table>

* Culture solution should be sterilized through 0.45 μm Eikikurodisk.
* Root-tips should be previously sterilized for 10 min (30°C) with antibiotics (see in the text).
* During the absorption, test tube should be covered with parafilm (American Can company) and be set in an incubator with 60° angle. If absorption is continued more than 30 min, depletion of the endogenous energy source will occur.
and subjected to radioactivity measurement with a liquid scintillation counter (Aloka LSC-651). The specific radioactivity of each phytosiderophore was then calculated.

Tracing of radioactivity with HPLC-radioanalyzer—A portion (within 20 \( \mu l \)) of the stock solution was injected to HPLC (JASCO) connected linearly with a Radioanalyzer (Aloka RLC-550) (Mori 1981a, b and 1982). Both the amount and radioactivity of each phytosiderophore were analyzed on one chart. In general, 10 mV-300 cpm with a 20-s time constant was adopted for the analytical conditions of the radioanalyzer. The analytical conditions for phytosiderophores with HPLC were modified slightly from the method of Mori et al. (1987). The main modifications were the use of two linearly connected columns instead of one column and a pH 2.75 buffer instead of a pH 2.80 buffer. These modifications promoted separation of the phytosiderophores. The details are described in Table 1.

Results

Location of phytosiderophore synthesis—As shown in Fig. 1, one phytosiderophore secreted from corn root was identified as deoxy-MA. Fig. 1 also shows its amount in the corn root segment. Deoxy-MA was identified using phytosiderophores secreted from ‘Minorimugi,’ which is known to secrete MA and deoxy-MA (Takagi et al. 1984). The first 0.5-cm root tip had twice as much deoxy-MA than the other parts when represented as an index of (peak area of deoxy-MA/peak area of serine), and its value decreased from the tip to the basal parts. This strongly suggests that phytosiderophore synthesis occurs in root tips. We therefore used the root tips of Fe-deficient plants in feeding experiments to survey for a precursor amino acid of phytosiderophores. Among the \(^{14}\)C-labeled amino acids and \(^{14}\)C-acetic acid fed to barley ‘Ehimehadaka No. 1,’ we discovered that only \([1-^{14}\)C]-Met could be quickly metabolized into phytosiderophores. Here we describe our findings on the metabolism of methionine as a precursor of phytosiderophores.

\([1-^{14}\)C]-Met incorporation into phytosiderophores of Hordeum vulgare L. c.v. Ehimehadaka No. 1.—Whether ‘Ehimehadaka No. 1’ secretes epiphydroxy-MA or hydroxy-MA had not been clarified in our previous study (Mori et al. 1987). However, in experiments using the authentic sample presented by Drs. Takagi et al., epiphydroxy-MA and a trace of hydroxy-MA were identified in the root washings of this plant (Fig. 2). Fig. 3 shows the results of the \([1-^{14}\)C]-Met feeding experiment. The radioactivity of sulfone, sulfoxide, deoxy-MA and Unk-1 appeared after 30-min feeding and increased with time. A trace of MA and a large amount of epiphydroxy-MA appeared after 2 h. It means that both MA and epiphydroxy-MA were produced after the synthesis of deoxy-MA.

\([1-^{14}\)C]-Met incorporation into phytosiderophores of Hordeum vulgare L. c.v. Minorimugi—MA secretion was found in ‘Minorimugi’ and its chemical structure was determined by Takemoto et al. (1978). The results obtained from the feeding experiment of \([1-^{14}\)C]-Met to the Fe-deficient root tips are shown in Fig. 4. Sulfone and sulfoxide, deoxy-MA and Unk-1 appeared after 30 min and their radioactivity levels increased with time. A large amount of MA appeared after 2 h, showing that MA as a metabolite after synthesis of deoxy-MA.

\([1-^{14}\)C]-Met incorporation into phytosiderophores of Avena sativa L.—Avena sativa was found to secrete avenic acid and its chemical structure was determined by Fushiya et al. (1980). Our plants secreted avenic acid and a trace of deoxy-MA and MA as shown in Fig. 5. The results of the experiment feeding \([1-^{14}\)C]-Met to Fe-deficient root tips are shown in Fig. 6. These root tips also produced epiphydroxy-MA. Avenic acid appeared after 30 min and also both sulfone and sulfoxide. Only traces were found of other amino acids, such as Asp, Thr, Ser, Asn, Glu, Gln. On the other hand, when \([1-^{14}\)C]-Met was introduced to Fe-sufficient root tips of \(Avena sativa\), only a trace of radioactivity was introduced into avenic acid even after 2-h feeding (Fig. 7). These results mean that avenic acid is produced from methionine, which is specific to Fe-deficient root
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Fig. 1

Fig. 1 Amount of deoxy-MA in each segment of the lateral roots from the tip to the basal parts of Fe-deficient corn. The analytical conditions of HPLC were as given in the previous paper (Mori et al. 1987).

Fig. 2 Secreted phytosiderophores from Fe-deficient barley 'Ehimehadaka No. 1.' HPLC was eluted with a buffer at pH 2.65 to separate between hydroxy-MA and epihydroxy-MA.

tips of Avena sativa.

[1-14C]-Met incorporation into phytosiderophore of Oryzae sativa L. c.v. Honenwase—The phytosiderophore in the root washings of Oryzae sativa was identified for the first time using HPLC as deoxy-MA as in the case of corn mentioned above (Fig. 8). Time-course study of [1-14C]-Met incorporation into Fe-deficient rice root tips showed that the radioactivity had entered deoxy-MA and its amount and specific activity increased linearly with time from 15 min up to 120 min (Fig. 9 and Table 3). Labeled sulfone and sulfoxide also increased with time. Unk-1 appeared after 30 min and increased with time. Unk-2 appeared after 2 h.

Metabolites from photosynthetic 14CO2 fixation in the root of 'Minorimugi'—After photosynthetic 14CO2 fixation, 'Minorimugi' secreted four 14C-labeled phytosiderophores, epihydroxy-MA, MA, deoxy-MA and avenic acid as shown in Fig. 10. The specific activity (dpm/nmol) of each labeled phytosiderophore was as follows: avenic acid 32.0, deoxy-MA 10.0, MA 14.6 and epihydroxy-
Fig. 3  HPLC-radioanalysis of the soluble fraction of root tips of Fe-deficient barley 'Ehimehada No. 1' fed [1-14C]-Met for 30 min and 120 min. Radioactivity was monitored with 300 cpm (10 mV) full scale and with a 20-s time constant.

Fig. 4  HPLC-radioanalysis of the soluble fraction of root tips of Fe-deficient barley 'Minorimugi' fed [1-14C]-Met for 30 min and 120 min. Radioactivity was monitored as given in Fig. 3.

Fig. 5  Secreted phytosiderophores from Fe-deficient Avena sativa. Avenic acid was the main phytosiderophore. Only traces of other amino acids were secreted.
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Fig. 6  HPLC-radioanalysis of the soluble fraction of root tips of Fe-deficient Avena sativa fed [1-14C]-Met for 30 min. Radioactivity was monitored as given in Fig. 3.

Fig. 7  [1-14C]-Met metabolism in Fe-sufficient root tips of Avena sativa. Only a trace of radioactivity was detected in avenic acid and MA even after 120 min treatment. Radioactivity was monitored as in Fig. 3.

MA 12.2. This means that avenic acid is the first phytosiderophore synthesized from the photosynthetates translocated from the top. Time-course labeled assimilates in the root tips of 'Minorimugi' are shown in Fig. 11. MA was labeled one day after 14CO2 fixation, but neither sulfone nor sulfoxide were labeled.

Discussion

Kawai et al. (1986) reported that when 15N (3%)-NO3 was supplied to Fe-deficient barley roots for 3 h and then the plant was transferred to 14N-NO3, the amounts of NO3-15N and MA-15N in the roots came to 7% and 1%, respectively, just after 3 h. After 15 h MA-15N in the roots increased to 1.7% while NO3-15N in the shoot was 1%. From these results, they assumed that MA might be produced in the root; it is not transferred to the root after synthesis in the shoot. Our previous morphological study using electron microscopy (Nishizawa and Mori 1987) also
Secreted deoxy-MA from Fe-deficient rice 'Honenwase.' Even under severe chlorosis, the amounts of amino acids secreted were higher than those of deoxy-MA. This phenomenon is different from that of *Avena sativa* (Fig. 5) or *Hordeum vulgare* (Fig. 10).

**Fig. 8**

Even under severe chlorosis, the amounts of amino acids secreted were higher than those of deoxy-MA. This phenomenon is different from that of *Avena sativa* (Fig. 5) or *Hordeum vulgare* (Fig. 10).

**Fig. 9**

HPLC-radioanalysis in the soluble fraction of root tips of Fe-deficient rice 'Honenwase' fed [1-14C]-Met for 15, 30, 60 and 120 min. Radioactivity was monitored at 1,000-cpm (10 mV) full scale with a 12-s time constant.

**Table 3**

Time course incorporation of radioactivity into deoxy-MA from [1-14C]-Met in the root-tips of Fe-deficient rice

<table>
<thead>
<tr>
<th>Time of absorption (min)</th>
<th>Radioactivity of deoxy-MA dpm ($\times 10^3$ dpm)</th>
<th>Specific activity of deoxy-MA dpm/nmol</th>
<th>% radioactivity incorporated into deoxy-MA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>85</td>
<td>84</td>
<td>0.31</td>
</tr>
<tr>
<td>30</td>
<td>193</td>
<td>214</td>
<td>0.70</td>
</tr>
<tr>
<td>60</td>
<td>372</td>
<td>465</td>
<td>1.34</td>
</tr>
<tr>
<td>120</td>
<td>571</td>
<td>860</td>
<td>2.06</td>
</tr>
</tbody>
</table>

Amount of deoxy-MA was calculated postulating that the sensitivity of deoxy-MA to OPA reagent was equal to MA, because we had not pure authentic deoxy-MA.
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Fig. 10  $^{14}$C-labeled phytosiderophores secreted from roots of Fe-deficient barley ‘Minorimugi’ collected the day after $^{14}$C-photosynthesis.  Details are given in the footnote in Fig. 11.

Fig. 11  Time course of $^{14}$C-labeled assimilates in root tips of ‘Minorimugi’.  $^{14}$CO$_2$-photosynthesis were conducted from 12:30 to 20:30 on March.  After that, 15 plants were cultured under light (12:30 pm–2:30 am) and dark (2:30 am–12:30 pm) conditions.  Root tips were cut off after secretion of phytosiderophores on the following dates: A: 1987.3.16, B: 1987.3.17 and C: 1987.3.19.  Radioactivity was monitored as given in Fig. 3.

suggested that phytosiderophores may be synthesized and/or stored in the specific grains surrounded with ribosomes in the cortex and/or the epidermal cell of the root tips of Fe-deficient barley ‘Ehimehadaka No. 1.’  Our findings with corn root (Fig. 1), this is the first report of a phytosiderophore identified for corn, strongly suggested that phytosiderophores are produced mainly in the root tips.

Based on the above three experimental results, we assumed that root tips of Fe-deficient Gramineae plants could be used to assay the precursor amino acids of phytosiderophores.

The Gramineae used secreted avenic acid, deoxy-MA, MA, hydroxy-MA and epihydroxy-MA.  Metabolic study using various $^{14}$C-labeled chemicals confirmed that these phytosiderophores are synthesized from methionine as the first precursor amino acid.  Met was metabolized into avenic acid (Avena sativa, Fig. 6 and ‘Minorimugi,’ Fig. 10) or into deoxy-MA (‘Honenwase,’ Fig. 9, ‘Ehimehadaka No. 1,’ Fig. 3 and ‘Minorimugi,’ Fig. 4).  Deoxy-MA is
metabolized into MA (‘Minorimugi,’ Fig. 4) or into epihydroxy-MA (‘Ehimehadaka No. 1,’ Fig. 3). Avenic acid was the first phytosiderophore synthesized from $^{14}$CO$_2$-photosynthetate translocated from the top (‘Minorimugi,’ Fig. 10).

The overall metabolic process of phytosiderophore synthesis is summarized as shown in Fig. 12, based on the above findings and considering the chemical structure of each compound. In a personal communication, Dr. Fushiya says that from the viewpoint of organic synthesis, deoxy-MA may be biometrically synthesized from avenic acid in one step. But he has not yet been successful with its synthesis under mild chemical conditions. The other steps after deoxy-MA should be completed by hydroxylation of 2-C and then 3-C positions. They may proceed enzymatically one by one. However, the route from methionine to avenic acid cannot be considered as one process from the enzymatic viewpoint.

If we suppose that avenic acid is composed of three methionine molecules as is shown in Fig. 13, methionine should first be decomposed into two parts at the S—C(4) bond (H$_3$C—S=CH$_2$—). Then an alkylating agent, C(4), must attack amino-N of the other methionine molecule. The

![Fig. 12](image.png)

**Fig. 12** A hypothetical metabolic pathway of phytosiderophore synthesis from methionine.

**Fig. 13** Hypothetical components of three methionine molecules in avenic acid. Component A cannot be derived from serine, because [U-$^{14}$C]-Ser was not incorporated into phytosiderophores in the roots of Fe-deficient ‘Ehimehadaka No. 1.’ The OH group of component C may be metabolically changed from the $\text{SH}_2$ group, after binding three molecules derived from methionine. * represents the border of the carbon skeleton derived from methionine. / represents alkylation reaction.
schematic mechanism is proposed in Fig. 13. The question arises of how the methionine molecule decomposes at the $S-C(4)$ bond by itself. Methionine is known to be a donor of the $-\text{CH}_3$ group after binding with the adenosyl group to the S position (Pullman 1963). This means that the C(5)$-S$ bond ($\text{CH}_3-S-\text{CH}_3-$) is easily decomposed in methionine catabolism. How the $S-C(4)$ bond in methionine is broken is not known. Therefore, we should consider the other metabolic products of methionine in the root tips as a real biological alkylating agent. That alkylating agent must break the $S-C(4)$ bond by itself. When [1-$^{14}$C]-Met is supplied as a precursor amino acid, sulfone and sulfoxide always appeared prior to phytosiderophore synthesis. Moreover both compounds always contained equal amounts of radioactivity. To prepare sulfone and sulfoxide in equal amounts from methionine by organic synthesis requires severe conditions. Under mild conditions, sulfoxide is the main product (Iselin 1961). Therefore, sulfone and sulfoxide which appear by [1-$^{14}$C]-Met feeding are considered to have been produced metabolically. They should not be non-biologically oxidized artifacts of the feeding and extraction process. Is there any possibility that sulfone and/or sulfoxide plays an important role as an alkylating agent in biological systems? There exist suggestive data to support this idea. For example, several chemical mutagens analogous to sulfone and sulfoxide appear in the list of alkylating agents used in plant breeding (Loveless 1966): ethylmethansulfonate (EMS), 1,4-di- (methane-sulfoxonoxy) butane (Myleran), (di)ethyl sulfate (DES), (di)methylsulfate (DMS), and methylmethansulfonate (MMS). They have the common group

\[
\begin{align*}
\text{O} \\
\text{O} \\
\text{S}-\text{CH}_3: \text{methanesulfonate}.
\end{align*}
\]

The next item to clarify is whether $^{14}$C-sulfone and/or $^{14}$C-sulfoxide can be directly incorporated into phytosiderophores or not.

We are deeply indebted to Professor Takagi and Dr. Kawai of Iwate University and to Dr. Nomoto of the Suntory Bioorganic Institute, for presenting us with authentic hydroxymugineic acid and ephedroxyhumugineic acid. We also thank Dr. Fushiya of Tohoku University for presenting us with authentic avenic acid A. Mr. Mihashi helped us with the analytical method of phytosiderophores with HPLC.

References


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