Rapid Paper

Reduction of Methionine Sulfoxide by Plants

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Methionine sulfoxide was reduced by plants such as rice, large crabgrass, oats, soybeans, Japanese dock and green amaranth, forming methionine. This reduction occurred rapidly but not completely. The sulfoxide was also reduced by an excess of ferrous chloride and methionine or in the presence of the boiled root system of rice plants.

A sulfoxide, isoprothiolane sulfoxide, has recently been found to be easily and exclusively reduced to isoprothiolane by a living rice plant.¹⁾ This is the first evidence for sulfoxide reduction by plants. Since isoprothiolane sulfoxide is an oxidative metabolite of isoprothiolane,²⁾ an interconversion between a thioether and the corresponding sulfoxide may occur in rice plants, in which the balance of the interconversion inclines to the thioether. More recently, the reduction of another sulfoxide, methionine sulfoxide, by various plants has also been studied in our laboratory and will be discussed here.

MATERIALS AND METHODS

L-[methyl-¹⁴C]Methionine (specific activity 60.4 mCi/mmol) was purchased from Amersham International (Buckinghamshire, UK). Its sulfoxide was obtained by storing the aqueous [¹⁴C]methionine (50 μ Ci/ml) at -20°C for a month³⁾ and identified by two dimensional co-thin-layer chromatography (*tlc*). Cold L-methionine, L-methionine sulfoxide and L-methionine sulfone (Sigma Chemical Co., St. Louis, U.S.A) were used as authentic compounds for co-*tlc*, in which precoated HPTLC silica gel plates (E. Merck, Darmstadt, West Germany) and solvent systems such as chloroform-methanol-water (70:35:10) and chloroform-methanol-28% ammonium hydroxide (70:40:5) were used. Cold compounds on *tlc*

plates were detected by UV (254 nm), iodine vapor and ninhydrin spray. Radioactivity on *tlc* plates was autoradiographically detected and determined by scraping appropriate regions into vials followed by liquid scintillation counting (*lsc*), for which an Aloka Liquid Scintillation Counting System 903 (Aloka Co., Tokyo, Japan) and a scintillation cocktail, ACS II®, (New England Nuclear, Boston, U.S.A) were used.

The plants used were rice, *Oryza sativa* L. cultivar Kinmaze, soybeans, *Glycine max* Merr., oats, *Avena sativa* L., large crabgrass, *Digitaria adscendens* Henr., Japanese dock, *Rumex japonicus* Houtt., and green amaranth, *Amaranthus viridis* L. After the root systems had been washed to dislodge any particles of soil, plants with 3~4 true leaves were water-cultured in a small tubes. [¹⁴C]Methionine sulfoxide (purity 94~99%) was added to the culture solution (0.1~1 μ Ci/0.25 ml/1~4 plants/tube) with a microsyringe.¹⁾ The plants were then placed in a growth chamber at 25°C. After 30 min or 1~2 hr, the plants were rinsed and extracted with 2 ml of chloroform-methanol (1:2). The extracts and the washes were combined with the culture solutions. [¹⁴C]Methionine and its sulfoxide in the combined solutions were analyzed by *tlc* and *lsc*.

RESULTS AND DISCUSSION

L-[¹⁴C]Methionine sulfoxide was quite stable in the absence of plants. However, it was transformed by the rice plants, forming a reduced product which was identified as [¹⁴C]methionine by two dimensional co-*tlc*. This reduction occurred rapidly but not completely, so that an equilibrium was attained within 1 hr (Table I). A gradual decrease of

TABLE I. REDUCTION OF [¹⁴C]METHIONINE SULFOXIDE BY RICE PLANTS

[¹⁴ C]Compound ^a	Amount or percentage of methionine			
	0	30	60	120 min
	(× 10 ² dpm/tube)			
Methionine	13.4	105.7	137.1	87.2
Methionine sulfoxide	1991.9	1505.8	706.8	449.3
Percentage	(%)			
methionine × 100				
methionine + methionine sulfoxide	0.7	9.1	16.3	16.3
¹⁴ C Recovery	100	80.4	42.1	26.8

^a [¹⁴C]Methionine sulfoxide was added to the culture solution in tubes (0.09 μ Ci/tube).

TABLE II. REDUCTION OF [14 C]METHIONINE SULFOXIDE BY 6 SPECIES OF PLANTS

Plant species	methionine $\times 100^a$
	methionine + methionine sulfoxide (%)
Blank (0) ^b	0.7
Rice (2)	9.1
Large crabgrass (4)	5.9
Oats (2)	14.1
Soybeans (1)	22.7
Japanese dock (4)	6.8
Green amaranth (4)	31.3

^a After 30 min, the amounts of methionine and its sulfoxide were determined by radioassay as described in the text.

^b Number of plants used.

TABLE III. REDUCTION OF [14 C]METHIONINE SULFOXIDE WITH COLD METHIONINE AND FERROUS CHLORIDE

Reducing reagent ^a	[14 C]methionine $\times 100^b$
	[14 C]methionine + [14 C]methionine sulfoxide (%)
Blank	1.2
Cold methionine (1,000 ppm)	90.3
Cold methionine sulfoxide (1,000 ppm)	3.0
Ferrous chloride (100 ppm)	2.6
Ferrous chloride (1,000 ppm)	26.9
Ferric chloride (1,000 ppm)	1.1

^a These reagents were added to water in tubes containing [14 C]methionine sulfoxide (0.1 μ Ci/0.1 ml/tube).

^b The amounts of [14 C]methionine and its sulfoxide were analyzed after 30 min reaction.

radioactivity recovery could be due to incorporation by the plants. Other plants such as large crabgrass, oats, soybeans, Japanese dock and green amaranth also reduced [14 C]-methionine sulfoxide to the corresponding thioether (Table II). Due to differences in their sizes, it was difficult to compare this activity among the plant species. No signifi-

FIG. 1. Thin-layer Chromatography of the Radioactivity Extracted from Living or Boiled Root System of Rice Plants and the Culture Solution 30 min after the Addition of [14 C]Methionine Sulfoxide to the Culture Solution.

The HPTLC silica gel plate was developed with chloroform-methanol-28% ammonium hydroxide (70:40:5).

cant formation of [14 C]methionine sulfone was observed throughout the experiment.

[14 C]Methionine sulfoxide was also reduced by an excess of ferrous chloride and cold methionine but not by ferric chloride or cold methionine sulfoxide (Table III). Such reducing reagents as ferrous chloride and methionine, if present in the plants, may contribute to the reduction of the sulfoxide. Indeed, the root system of plants seems to contain more or less amounts of ferrous compounds and methionine.^{4,5} Reduction of [14 C]methionine sulfoxide was also observed in the presence of the boiled root system of rice plants (Fig. 1). This may be by chemical reduction by reducing reagents present in the plants.

As the first evidence for the reduction of sulfoxides by plants, we have reported that a living rice plant can reduce isoprothiolane sulfoxide rapidly and almost exclusively to isoprothiolane,¹ the sulfur atoms of which are included in its dithiolane ring so that isopro-

thiolane sulfoxide can be more easily reduced than non-cyclic sulfoxides.⁶⁾ The reduction of methionine sulfoxide in the current paper suggests strongly that plants can reduce various types of sulfoxides to the corresponding thioethers.

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