Uptake and Metabolism of $\text{NaH}^{35}\text{SO}_3$ in Rice and Barley Seedlings

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Comparative laboratory experiments regarding the effects of NaHSO_3 on growth and the incorporation of $\text{NaH}^{35}\text{SO}_3$ into rice and barley seedlings were examined. Both rice and barley seedlings showed much lower growth above 10^{-3}M ($=\text{mol/l}$) concentration of NaHSO_3 in the medium, than the control. The reduced ratio to the control was higher in barley than in rice.

Total incorporation of $\text{NaH}^{35}\text{SO}_3$ declined with an increase of NaHSO_3 during culture. Rice incorporated $\text{NaH}^{35}\text{SO}_3$ into the tissues much more than barley at late phase of culture and pooled it as an inorganic form without translocation to organic metabolites. However in barley, the fixation of $\text{NaH}^{35}\text{SO}_3$ in the ethanol insoluble and cationic fraction increased remarkably during culture. In the two plant tissues at 10^{-3}M , the ^{35}S distribution into sulfur amino acids in the cationic fraction was lower than that of control. More than 80% of the radioactivity was occupied by sulfur amino acids in the protein hydrolysates similarly in rice and barley.

Key Words: sodium hydrogensulfite solution, rice, barley, metabolism, $\text{NaH}^{35}\text{SO}_3$ uptake, sulfur amino acid

1. Introduction

Sulfur dioxide (SO_2) is a major pollutant in industrialized areas, and can damage plant vegetation. Much of the published work, however, has been concerned with the analysis of visible damage and research for alleviating factors. Plants have long been known to differ in their sensitivities to SO_2 pollution, and several lists of the susceptibility of species to this pollutant have been published¹⁾⁻³⁾.

On the other hand sulfur is an essential element of plants and it is a major component of amino acids, proteins and other cellular constituents. Some of the SO_2 absorbed into plant tissues is metabolized⁴⁾⁻⁶⁾. While there have been studies of the metabolism of SO_2 in individual species, there have been no comparative analyses of SO_2 metabolism in species of differing sensitivities to the gas.

Rice is regarded as a comparatively resistant

plant to SO_2 , and barley as a sensitive plant⁷⁾. In this report, comparative laboratory experiments regarding the effects of NaHSO_3 on growth and the incorporation of $\text{NaH}^{35}\text{SO}_3$ into rice and barley seedlings are presented.

Many authors have exposed plants to SO_2 , however the use of $^{35}\text{SO}_2$ creates many difficulties when used for treatments. In this experiment a buffered sulfite solution (NaHSO_3 and $\text{NaH}^{35}\text{SO}_3$) was used instead of gaseous SO_2 ^{8),9)}.

2. Materials and Methods

2.1 Plant materials

Seeds of rice (*Oryza sativa* L., var. Yamabuki) and barley (*Hordeum vulgare* L., var. Akashin-riki) were soaked in 10% H_2O_2 for 15 min, then germinated for 20 h at 25°C in the light. The germinated seeds were cultured at 25°C for about 2 weeks with 16 h photoperiods in the medium shown in Table 1. The media contained different

Table 1 Composition of culture solution*

Rice	{	NaNO ₃	2.5 mM	KCl	1.5 mM
				KH ₂ PO ₄	0.5
	{	NH ₄ NO ₃	2.0	CaCl ₂ ·2H ₂ O	1.0
				MgSO ₄ ·7H ₂ O	0.5
Barley	{	NaNO ₃	3.5	Fe	3 ppm
				Mn	0.5
	{	NH ₄ NO ₃	1.0	B	0.5

* Concentration of ions other than nitrogen sources are the same in rice and barley.

concentrations of NaHSO₃ in 0.01 M (=mol/l) Tris-maleate-KOH buffer (pH 7.0) and were renewed every 3 d. At the appropriate time, seedlings (10–20 shoots) were collected and the average fresh weight and height were determined.

2·2 Incorporation of NaH³⁵SO₃ and fractionation

The germinated seeds were transferred to a plastic tray with 600 ml of culture solution containing NaH³⁵SO₃ (specific activity, 636.4 MBq/mmol, 0.37 MBq/ml solution), grown for 11 days and the incorporation of NaH³⁵SO₃ into the seedlings was determined. The shoots were collected and homogenized with 50 ml of 80% boiling ethanol containing 0.04 M *N*-ethylmaleimide¹⁰⁾. The suspension was centrifuged and separated into soluble and insoluble fractions. The extraction was repeated 3 times. The combined extract was passed through an ion exchange column of Amberlite IR-120 resin (H⁺) to eliminate cationic substances. The adsorbed cationic substances were eluted from the column with 80 ml of 2 M NH₄OH, and represented the free amino acid fraction¹¹⁾. The radioactivity of ³⁵S in the 3 fractions was measured by a gas flow counter.

All experiments were repeated 2 to 3 times, and the figures and tables show the data from a representative experiment due to no fundamental difference in the metabolic pattern between the ex-

periments.

3. Results and Discussion

Figure 1 shows the growth pattern of rice and barley cultured in the medium containing NaHSO₃, as average fresh weight of one shoot. NaHSO₃ concentration in the media varied from 0 (control) to 2×10⁻³ M. The growth patterns of rice and barley seedlings were very similar during the culture. No significant inhibition of growth was observed below 10⁻⁴ M. A slight decline was found at 5×10⁻⁴ M, especially in the late phase of culture compared to the control plant. However, the growth reduction in rice and barley seedlings was not paralleled with an increase of NaHSO₃ concentration in the medium. A clear reduction of the fresh weight at the level of 10⁻³ M began to appear after 2 d of culture for barley and after 4 d for rice. Both rice and barley seedlings showed much lower growth above 10⁻³ M than the control. The reduced ratio to the control was higher in barley than in rice. The result suggested a possibility that rice is more resistant than barley to NaHSO₃ at young seedling stage.

Visible damage, especially chlorosis in the top area of the leaf was found at 10⁻³ M after 8 d in rice and barley seedlings. At 2×10⁻³ M, seedlings were completely damaged and the roots became black and did not grow after 6 d.

Regarding shoot height, a more similar growth pattern was observed between rice and barley seedlings with an increase of NaHSO₃ in the medium (Fig. 2). A distinct reduction was found above 5×10⁻⁴ M concentration like the fresh weight. These results in growth patterns inducing visible injury showed that there is a similar threshold value of damage between 5×10⁻⁴ M and 10⁻³ M of NaHSO₃ in rice and barley, similar to SO₂ fumigation¹²⁾.

Taniyama has reported in rice without showing

Fig. 1 Growth pattern in fresh weight of rice and barley seedling.

The concentration of NaHSO_3 varied from 0 (control) to 2×10^{-3} M. Day 0 is at the end of a 20 h inhibition at 25 °C. Values are expressed as the fresh weight of one shoot.

Fig. 2 Growth pattern in shoot height of rice and barley seedling.

Values are expressed as the shoot height of one shoot. Others are the same as shown in Fig. 1.

visible injury, that the physiological inhibition of photosynthesis and respiration increased gradually with the increasing SO_2 fumigation¹³⁾. Though NaHSO_3 at 10^{-3} M concentration induced a clear growth reduction here, some NaHSO_3 would be metabolized into sulfur compounds containing toxic products in the plant tissues. Next, a $\text{NaH}^{35}\text{SO}_3$ incorporation experiment into the seedlings cultured at 10^{-3} and 10^{-4} M concentrations showing visible and invisible growth reduction, respectively, was performed.

Table 2 shows the incorporation of $\text{NaH}^{35}\text{SO}_3$ into rice and barley seedlings cultured at 10^{-4} M

Table 2 Incorporation of $\text{NaH}^{35}\text{SO}_3$ into rice and barley seedlings

Plant NaHSO_3	Culture days			
	4	6	8	11
Rice				
0	2 513	6 979	21 228	40 989
10^{-4} M	950	5 260	21 149	40 139
10^{-3} M	1 259	3 399	9 150	21 759
Barley				
0	3 049	6 101	12 000	21 499
10^{-4} M	2 899	5 199	9 298	16 650
10^{-3} M	1 490	3 799	6 104	6 849

Values are expressed as $\text{cpm} \times 10^{-2}$ per 10 shoots.

and 10^{-3} M of NaHSO_3 . Total incorporation of $\text{NaH}^{35}\text{SO}_3$ declined with an increase of NaHSO_3 concentration in the medium during culture. In particular, the incorporation decreased largely at 10^{-3} M of both seedlings, in which a clear growth reduction was found, whilst a little difference between control and 10^{-4} M. This incorporation pattern was more distinctive in rice, showing higher $\text{NaH}^{35}\text{SO}_3$ incorporation especially at late phase of culture, than in barley.

Table 3 shows the distribution of incorporated $\text{NaH}^{35}\text{SO}_3$ activity in ethanol soluble, insoluble and cationic fractions of rice and barley seedlings. The most characteristic difference between rice and barley was found in the metabolically fixed activity of $\text{NaH}^{35}\text{SO}_3$. In barley, the fixation of $\text{NaH}^{35}\text{SO}_3$ in the ethanol insoluble and cationic fraction, showing much higher values than that of rice at each carrier level, increased remarkably during culture. On the contrary, in rice, the low fixation into the ethanol insoluble and cationic fraction did not increase so remarkably. At 11 d culture, the percentage of fixed activity (ethanol insoluble + cationic fraction/total incorporated activity) in barley reached 36.4% (control), 31.8% (10^{-4} M), and 25.2% (10^{-3} M). However in rice, the percentages were, 13.3% (control),

Table 3 Distribution of $\text{NaH}^{35}\text{SO}_3$ activity in rice and barley seedlings

Culture days		4			6			8			11		
Plant	Fraction	Ethanol soluble fraction	Ethanol insoluble fraction	Cationic fraction	Ethanol soluble fraction	Ethanol insoluble fraction	Cationic fraction	Ethanol soluble fraction	Ethanol insoluble fraction	Cationic fraction	Ethanol soluble fraction	Ethanol insoluble fraction	Cationic fraction
Rice													
0		1 942	501	70	5 914	905	160	18 684	2 303	341	35 620	4 755	614
10^{-4} M		1 648	251	51	4 566	599	95	19 098	1 840	211	35 798	3 823	518
10^{-3} M		1 140	99	20	3 050	298	51	8 445	603	102	19 910	1 653	196
Barley													
0		2 159	750	140	4 197	1 703	201	8 340	3 204	456	13 674	6 600	1 225
10^{-4} M		2 268	501	130	3 936	1 102	161	7 030	1 850	418	11 322	4 695	633
10^{-3} M		1 250	200	40	2 960	699	140	4 798	1 105	201	5 123	1 404	322

Values are expressed as $\text{cpm} \times 10^{-2}$ per 10 shoots.

10.9% (10^{-4}M), and 8.3% (10^{-3}M) suggesting that rice incorporated $\text{NaH}^{35}\text{SO}_3$ into the tissues much more than barley, and pooled it mostly as an inorganic form without translocation to organic metabolites (free amino acids, proteins and other sulfur compounds) during culture. The slow metabolic turnover of ^{35}S , especially to toxic sulfur compounds, indicates a resistant response of rice to NaHSO_3 . A comparatively high fixed ratio of 22.7% at 4 d in the control of rice did not increase, further. The tendency showing no increase in the fixed ratio was more remarkable at 10^{-4} and 10^{-3}M than the controls.

Godzik and Liskens reported that SO_2 fumigation increases the total amount of free amino acids in bean leaves¹⁴⁾. Although, the addition of NaHSO_3 did not induce an increase of ^{35}S activity distribution in the cationic fraction in this experiment, some changes of ^{35}S distribution in sulfur amino acids were considered. The distribution pattern into sulfur amino acids was examined using the seedlings cultured for 8 days at 10^{-3}M NaHSO_3 .

The free radioactive amino acids in the cationic fraction after removing ammonia dissolved in 5 ml of citrate buffer (pH 2.2) and the composition was fractionated with an automatic amino acid analyzer. The hydrolysates in the ethanol insoluble fraction containing proteins were also analyzed after acid decomposition¹¹⁾.

Five radioactive peaks appeared in the chromatographic pattern of 2 fractions by amino acid analyzer and 2 of these were not identified. Other 3 peaks were identified as sulfur amino acids by the chromatography of standard amino acids. The sulfur amino acids were collected, the radioactivity was determined and the incorporated ratio is shown in Table 4. No unusual peak was found in either plant cultured at 10^{-3}M compared with the control. Most of the ^{35}S activity in 2 fractions,

Table 4 Distribution of ^{35}S activity into sulfur containing amino acids of cationic and ethanol insoluble fractions in rice and barley seedling cultured for 8 days

	NaHSO_3	Cationic fraction (%)	Ethanol insoluble fraction (%)
Rice	0	73.4	80.2
	10^{-3}M	66.4	82.2
Barley	0	66.8	82.5
	10^{-3}M	60.4	84.1

Values are expressed as per cent of total radioactivity in each fraction.

especially in the ethanol insoluble fraction, were occupied with sulfur amino acids. In the 2 seedlings at 10^{-3}M , the ^{35}S distribution into sulfur amino acids in the cationic fraction was lower than that of control.

The author supposed that toxic NaHSO_3 might be metabolized to non-toxic metabolites like sulfur amino acids as a resistant reaction to SO_2 in rice. However, such a distinctively higher translocation of $\text{NaH}^{35}\text{SO}_3$ at 10^{-3}M did not occur even in rice. Rice had an ability firstly to accumulate toxic NaHSO_3 as an inorganic form without rapid translocation to usual organic metabolites. The distribution of ethanol insoluble fraction was the same level in rice and barley. More than 80% of $\text{NaH}^{35}\text{SO}_3$ was occupied by sulfur amino acids in the protein hydrolysates. $\text{NaH}^{35}\text{SO}_3$ is very stable when incorporated into proteins of plant tissues, compared to labile metabolism at the inorganic level. The use of $\text{NaH}^{35}\text{SO}_3$ in experiments of SO_2 metabolism of plants will be beneficial. However, further comparative studies of SO_2 metabolism using many kinds of sensitive and resistant plants grown in controlled $\text{NaH}^{35}\text{SO}_3$ environments are necessary.

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要 旨

NaH³⁵SO₃ のイネおよびオオムギ幼植物中への取込みと代謝

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亜硫酸ガス傷害に比較的強いといわれるイネと、弱いといわれるオオムギ幼植物を供試して放射性亜硫酸水素ナトリウム (NaH³⁵SO₃) を経根的に取り込ませ、両者の反応性の違いを調べた。培養液中の NaHSO₃ 濃度が 5×10^{-4} M 以下では両植物とも生長は阻害されなかった。 10^{-3} M 以上で強く阻害され、その程度はオオムギの方が大きかった。

NaH³⁵SO₃ (0.37 MBq/ml) の shoot への取込みを調べた結果、両植物とも 10^{-4} M では対象と大きな違いはなかったが、 10^{-3} M では低下した。全取込み量は、栽培後半にイネがオオムギより約 2 倍高く、その大部分は無機態でプールされていた。取り込まれた NaH³⁵SO₃ の他硫黄化合物への代謝を調べると、オオムギでは、アルコール溶・カチオン性画分、アルコール不溶画分中の有機化合物への代謝の割合が高く、11日後の 10^{-3} M で 25.2% を占めていた。そのときイネでは 8.3% と低かった。両画分中の含硫アミノ酸への代謝を調べると、カチオン性画分では 60 数% と対象よりやや少なく、アルコール不溶画分では 80% 以上を占め、対象よりやや多い傾向が両植物で認められた。