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Relationship Between Uptake of Mercury Vapor by Mushrooms and its Catalase Activity

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MINAGAWA et al. (1980) reported that mushrooms planted in water have the ability to accumulate mercury vapor. MAGOS et al. (1974) reported that catalase activity of human blood cells incubated with 3-amino-1,2,4-triazole in the presence of methylene blue decreased and the uptake of mercury vapor by the erythrocytes also decreased. In animals, erythrocytes, lung, and liver homogenates from acatalasemic mice had decreased abilities to take up mercury vapor when compared with those of normal mice (OGATA et al. 1978). In human acatalasemic erythrocytes, the uptake of mercury was also decreased as compared with that in normal erythrocytes (OGATA et al. 1979). These results suggest that catalase has an important role in the uptake of metallic mercury vapor in animals.

This communication deals with the uptake of mercury vapor by mushroom (Shiitake) artificially grown on an oak tree and the uptake in vitro by catalase extracts prepared from mushroom, Hay Bacillus and spinach.

MATERIALS AND METHODS

Preparation of samples, chemicals and analysis: Mushroom used in the exposure study were those grown artificially on oak (Kunugi) tree. Hay Bacillus was obtained from Natto (fermented soy-beans). Fresh mushroom, Natto and spinach used in in vitro experiments were purchased from commercial sources.

Chemicals used were all reagent grade. Measurement of total mercury has been described previously (OGATA et al. 1978, 1979). Catalase activity was estimated by the perborate method (FEINSTEIN 1949). Protein was measured by Lowry's method.

Exposure of mushroom to mercury vapor: Fresh mushroom growing on oak tree were placed in a glass vessel and exposed to mercury vapor (1.4 mg Hg/m^3) for 11 days. Mushroom were cut into small pieces and homogenized with water using a glass homogenizer with a Teflon pestle. The homogenate was used for measurement of mercury content.

Preparation of crude catalase extract: Mushrooms were homogenized with water using Polytron (Kinematica GmbH). After ultra-

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sonication of the homogenate, it was centrifuged at 2,500 x g for 10 min and filtered through filter paper (Whatman 41). M/15 phosphate buffer (pH 6.8) was added to the filtrate to give a final concentration of M/60.

Catalase extract from Hay Bacillus was prepared by the modified method of HERBERT (1948). Hay Bacillus was collected from Natto and lysed with lysozymes in 0.5% NaCl at 37°C for 1 h. After salting out the lysate with $(NH_4)_2SO_4$, the resulting precipitates were dissolved in 0.005N NaOH and filtered. After neutralizing the filtrate with 0.01N HCl, 1/3 volume of M/15 phosphate buffer (pH 6.8) was added to it.

Catalase extract from spinach was prepared by the modified method of GALSTON (1951). Spinach leaves were homogenized with acetone at -15°C and filtered. The residue was extracted with 0.1M Na_2HPO_4 and half saturated with solid $(NH_4)_2SO_4$. The precipitate was redissolved in M/15 phosphate buffer (pH 6.8) and filtered.

Preincubation of catalase extract with catalase inhibitor: In order to inhibit catalase, potassium cyanide, sodium azide or 3-amino-1,2,4-triazole with 4 mM ascorbic acid was added to a reaction mixture and preincubated for 1 h at 37°C.

Incubation: 3 mL of preincubated mixture was placed in the main chamber of a 15 mL Warburg flask with 0.1 mL of metallic mercury in a side-arm and with or without 0.1 mL of 3% hydrogen peroxide with shaking at 80 cycles/min as described previously (OGATA et at. 1978, 1979). After incubation, mercury content of the reaction mixture was measured.

RESULTS

TABLE 1. Mercury level in mushroom exposed to mercury vapor

days	Mercury concentration (μg/g)[1)]
0.5	0.4 ± 0.1
1.5	1.3 ± 0.1
2.5	2.0 ± 0.1
3.5	2.9 ± 0.1
4.5	3.4 ± 0.1
5.5	3.5 ± 0.1
10.5	4.6 ± 0.2

1) Wet weight basis, mean ± S.D.

Exposure experiment to mercury vapor: Mushroom were exposed to mercury vapor and the mercury content was measured at the plait of the cap (Table 1). Mercury concentration in the exposed mushroom

TABLE 2. In vitro mercury uptake by crude catalase extracts from mushroom, Hay Bacillus and spinach, and the effect of catalase inhibitors on the uptake.

Species	Addition				Mercury uptake[1]		Catalase activity[1]	
	H_2O_2	KCN	NaN_3	AT[2]	ng/mg protein	Ratio to (+)H_2O_2	Decrease (%)	PU/mg protein
Mushroom (Shiitake)	+	-	-	-	1160 ± 84.0	1.00	0	1.02 ± 0.10
	-	-	-	-	420 ± 5.7	0.36	64	1.00 ± 0.20
	+	+[3]	-	-	460 ± 16.0	0.40	60	0.15 ± 0.01
	+	-	+[3]	-	nd[4]	0.00	100	nd[4]
	+	-	-	+	70 ± 7.0	0.06	94	0.01 ± 0.01
Hay Bacillus	+	-	-	-	280 ± 9.4	1.00	0	0.38 ± 0.02
	-	-	-	-	100 ± 8.0	0.37	63	-
	+	+[5]	-	-	70 ± 18.3	0.25	75	-
	+	-	+[5]	-	10 ± 2.2	0.04	96	-
Spinach	+	-	-	-	990 ± 98.0	1.00	0	1.09 ± 0.20
	-	-	-	-	350 ± 30.0	0.35	65	-
	+	+[6]	-	-	520 ± 60.0	0.53	47	-
	+	-	+[6]	-	nd[4]	0.00	100	-

1) mean ± S.D., 2) 10 mM of 3-amino-1,2,4-triazole (AT) with 4 mM of ascorbic acid, 3) 10 mM,
4) non-detectable, 5) 1.33 mM, 6) 2 mM

increased depending on the days of exposure and reached a steady state after ten days. The concentration in ten days was 4.5 μg/g (wet weight basis).

Mercury uptake in vitro by mushroom catalase extract: Catalase extract prepared from mushroom took up mercury vapor (Table 2). A 64% decrease in mercury uptake was found without bydrogen peroxide as compared with the mercury uptake with hydrogen peroxide. The uptake of mercury was inhibited by potassium cyanide, sodium azide and 3-amino-1,2,4-triazole. The uptake of mercury paralleled the catalase activity. 10 mM of potassium cyanide gave a 60% decrease in mercury uptake; 10 mM of sodium azide inhibited the uptake of mercury completely; and 10 mM of 3-amino-1,2,4-triazole gave a 94% decrease in mercury uptake.

Mercury uptake in vitro by Hay Bacillus catalase extract: Similar results were obtained with Hay Bacillus (Table 2). Catalase extract prepared from Hay Bacillus gave a 63% decrease in mercury uptake without hydrogen peroxide as compared with the mercury uptake with the hydrogen peroxide. 1.33 mM of potassium cyanide gave a 75% decrease in mercury uptake, and 1.33 mM sodium azide inhibited it completely.

Mercury uptake in vitro by spinach catalase extract: Similar results were also obtained with spinach (Table 2). Catalase extract prepared from spinach gave a 65% decrease in mercury uptake without hydrogen peroxide as compared with the mercury uptake with hydrogen peroxide. 2 mM of potassium cyanide gave 47% decrease in mercury uptake, and 2 mM sodium azide inhibited it completely.

DISCUSSION

The mercury concentration in mushroom taken in the fields is higher than in other plants (SCHLENZ et al. 1974). This might suggest that the mushrooms absorb metallic mercury in air. If this is so, the level of mercury in the mushroom can be used as an indicator of mercury pollution in the environment.

In this experiment, mushroom grown on an oak tree took up metallic mercury vapor in the air (Table 1). The uptake of mercury by a catalase extract prepared from mushroom was observed and inhibited by addition of potassium cyanide and sodium azide, both of which are inhibitors of catalase and peroxidase, and also by 3-amino-1,2,4-triazole which is a specific inhibitor of catalase. Mercury uptake paralleled the change in catalase activity. Similar results were obtained with Hay Bacillus and spinach (Table 2).

These results suggest that catalase has an important role in the uptake of metallic mercury vapor in the plant.

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