Short Communication

Mutagenicity of Trioses and Methyl Glyoxal on *Salmonella typhimurium*

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It has been reported by many workers that the pyrolysates of some sugars show mutagenicity or enhancing activity on some mutagens.^{1~6)} However, there has been no report on the mutagenicity of intact sugar itself. We previously reported on the mutagenicities of many substances mostly contained in foods.^{7,8)} In the course of these studies, we found out that, among the sugars tested, only glyceraldehyde and dihydroxyacetone showed high mutagenicity on *Salmonella typhimurium* TA 100 without metabolic activation. Therefore we made further studies about those compounds having similar structures to these sugars, and methyl glyoxal and hydroxyacetone were also found to show mutagenicity.

This report deals with the mutagenicities of trioses, methyl glyoxal, hydroxyacetone and their related compounds on *Salmonella typhimurium* TA 100.

The mutagenicity was tested according to the method of Ames *et al.* with some modifications.⁹⁾ The histidine-requiring strains of *Salmonella typhimurium* were kindly supplied by Dr. B. N. Ames. The liver microsomal fraction was prepared from rats which had been injected with PCB as described by Ames *et al.*¹⁰⁾ The mutagenicity test was performed at five concentration levels for each sample, but only results giving the highest potency are shown in this paper.

DL-Glyceraldehyde and dihydroxyacetone were purchased from Sigma Chemicals Co. Methyl glyoxal (40% in water) and hydro-

xyacetone (50% in methanol) were obtained from Nakarai Chemicals Co. All other reagents used were of guaranteed grade.

The mutagenicity of trioses on *Salmonella typhimurium* TA 100 was tested with and without S-9 Mix.

As shown in Table I, it was found that glyceraldehyde ($\text{HOCH}_2\text{CH}(\text{OH})\text{CHO}$) and dihydroxyacetone ($\text{HOCH}_2\text{COCH}_2\text{OH}$) were highly mutagenic on this strain without activation with the S-9 Mix. There was no effect on the mutagenicity by pretreatment of these samples with S-9 Mix.

From these results, we were interested to know whether other sugars or their analogues would show mutagenicity or not. The results are also shown in Table I. Methyl glyoxal (CH_3COCHO) was found to have high mutagenicity and hydroxyacetone ($\text{CH}_3\text{COCH}_2\text{OH}$) was observed to be moderately mutagenic. Malonaldehyde (OCHCH_2CHO) showed slight mutagenicity but glycerol,

TABLE I. MUTAGENICITY OF TRIOSES AND THEIR RELATED COMPOUNDS ON *Salmonella typhimurium* TA 100

Cells of *Salmonella typhimurium* TA 100 were treated at 37°C for 60 min with samples of various concentrations without S-9 Mix, then poured on to an His-deficient culture medium. The plates were incubated at 37°C for 2 days and the induced His⁺ revertant colonies were counted. The mean number of spontaneous revertants (120) was subtracted.

	Conc./plate (μg)	Mutagenicity (Revertants/plate)
DL-Glyceraldehyde	100	650
Dihydroxyacetone	200	294
Erythrose	500	75
Xylose	500	53
Ribose	500	25
Glycerol	500	51
Hydroxyacetone	400	294
Acetone	500	25
Trimethylene glycol	400	46
Methyl glyoxal	30	1,530
Malonaldehyde	200	110
Pyruvic acid	200	74
Propylene oxide	400	522
Glycidol	300	1,126
(2,3-Epoxy-1-propanol)		

erythrose, xylose, ribose, pyruvic acid and trimethylene glycol ($\text{HOCH}_2\text{--CH}_2\text{--CH}_2\text{OH}$) did not show any mutagenicity with or without S-9 Mix.

The present observation will be the first to show the mutagenicity of glyceraldehyde, dihydroxyacetone, methyl glyoxal and hydroxyacetone.

It has been reported that some epoxy compounds such as glycidol ($\text{H}_2\text{C--CH--CH}_2\text{OH}$)

and propylene oxide ($\text{H}_2\text{C--CH--CH}_3$) show

mutagenicity on *Salmonella typhimurium*,¹¹⁾ which seem to be an analogue to these trioses or methyl glyoxal. Our experiments also showed that both of these epoxides had high mutagenicity on *Salmonella typhimurium* TA 100 (Table I).

Glyceraldehyde, dihydroxyacetone and methyl glyoxal showed weak mutagenicity on *Salmonella typhimurium* TA 98 but there was no activity on other *Salmonella* strains (TA 1535, TA 1536, TA 1538).

Figure 1 shows the dose response curves of methyl glyoxal, glyceraldehyde, dihydroxyacetone and hydroxyacetone on *Salmonella typhimurium* TA 100.

The maximum potencies of methyl glyoxal and glyceraldehyde were found at concentrations of 50 and 80 μg per plate.

Figure 2 shows the heat modification to the mutagenicity of these compounds.

The mutagenicities of glyceraldehyde, dihydroxyacetone and hydroxyacetone were markedly activated by heating at 60°C for 10 min. At temperatures above 60°C, the detected potency was low. Methyl glyoxal, on the other hand, was not so much affected by heat-treatment. No activating effect by heating was observed on glycidol or propylene oxide.

Trioses are known as intermediates in the pathway of glycolysis and they are always contained in living cells. By this study, it became clear that they have specific mutagenicities.

Omura *et al.* have found that triose reductone (HOCH=C(OH)CHO) shows muta-

FIG. 1. Dose Response Curves of Dihydroxyacetone, Glyceraldehyde, Methyl Glyoxal and Hydroxyacetone.

Cells of *Salmonella typhimurium* TA 100 were treated at 37°C for 60 min with samples of various concentrations, then poured on to an His-deficient culture medium. The plates were incubated at 37°C for 2 days and the induced His⁺ revertant colonies were counted. The number of spontaneous revertants (120) was subtracted. The samples were: ○, methyl glyoxal; ●, glyceraldehyde; △, dihydroxyacetone; ▲, hydroxyacetone.

FIG. 2. Effect of Heat-treatment on the Mutagenicity of Glyceraldehyde, Dihydroxyacetone, Methyl Glyoxal and Hydroxyacetone.

Each of the aqueous solutions (0.1 ml) containing either 30 μg of methyl glyoxal, 80 μg of glyceraldehyde, 200 μg of dihydroxyacetone or 400 μg of hydroxyacetone was added to 0.25 ml of 0.1 M phosphate buffer, pH 7.4. They were heated at various temperatures for 10 min. Cells of *Salmonella typhimurium* TA 100 were treated with these samples for 60 min and the mutagenicity then assayed as described in the text and Table I. ○, methyl glyoxal; ●, glyceraldehyde; △, dihydroxyacetone; ▲, hydroxyacetone.

genicity and that this mutagenicity is remarkably activated by the presence of Cu^{2+} on *Salmonella typhimurium*.¹²⁾ The triose reductone will have similar reactivity to those of the four compounds mentioned above.

Szent-Györgi hypothesized that methyl glyoxal will play an important part as a growth control and that glyoxalase will play as a growth activator.¹³⁾ This hypothesis attracts our attention from the view-point of carcinogenesis and anti-carcinogenesis. It may be interesting to do further studies on the mutagenicity of methyl glyoxal and trioses with this hypothesis.

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