Mutagenesis vol. 22 no. 1 pp. 43–47, 2007
Advance Access Publication 14 December 2006
doi:10.1093/mutage/gel057

Mutagenicity of UV-irradiated maltol in *Salmonella typhimurium*

Mie Watanabe-Akanuma[1,*], Yohei Inaba[2] and Toshihiro Ohta[2]

[1]Kureha Corporation Biomedical Research Laboratories, 3-26-2, Hyakunin-cho, Shinjuku, Tokyo 169-8503, Japan and [2]School of Life Science, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

We investigated the photomutagenicity of maltol (3-hydroxy-2-methyl-4*H*-pyran-4-one) in bacterial cells. Maltol has a caramel-butterscotch odour and is used as a food additive to impart flavour to bread and cakes. Unirradiated maltol was not mutagenic up to 5 mg/plate in the Ames test. When maltol was irradiated with either UVA (a black light, 320–400 nm, 230 μW/cm^2) for 5–30 min or UVC (a germicidal lamp, 610 μW/cm^2) for 3 min in sodium phosphate buffer (pH 7.4) prior to the exposure of bacterial cells, it was mutagenic to *Salmonella typhimurium* TA100, TA104 and TA97. Mutagenic activation of maltol by UVA-irradiation was more evident in neutral and alkaline conditions (pH 7.0–9.0) than in acidic conditions. On the other hand, photomutagenicity was not observed when maltol was irradiated with UVA in 100 mM NaCl solution or water. The mutagenic photoproduct was stable for at least 60 min after UVA-irradiation. However, addition of thiol compounds (cysteine or glutathione) to the UVA-irradiated maltol diminished the mutagenicity. Mutational spectrum analysis revealed that the predominant base-substitutions induced were G:C→T:A transversions and G:C→A:T transitions. An increase of 8-hydroxydeoxyguanosine formation in salmon sperm DNA exposed to maltol and UVA *in vitro* was detected by HPLC-ECD, but it was too small to explain the photomutagenicity. We are considering the formation of DNA adducts as the photomutagenic mechanism.

Introduction

Kojic acid (5-hydroxy-2-hydroxymethyl-4*H*-pyran-4-one), which had been used as a food additive and in cosmetics, was withdrawn from the list of existing food additives in Japan in 2003, because the possibility of involvement of genotoxicity of kojic acid in mouse hepatoma development could not be excluded. Since there were contradictory reports on the mutagenicity of kojic acid (1), collaborative studies had started using samples with different purity (2). We investigated the contribution of possible photomutagenicity of kojic acid with *Salmonella* reverse mutation assay and found no enhancement of mutagenicity of kojic acid by UVA-irradiation (M. Watanabe-Akanuma, unpublished data). On the other hand, we unexpectedly found photomutagenic property of maltol (3-hydroxy-2-methyl-4*H*-pyran-4-one), a structurally similar compound. Maltol is a naturally occurring compound found in bark of larch trees, chicory oils and roasted malt. It has a caramel-butterscotch odour and is used as a flavouring to impart 'freshly baked' odour and flavour to bread and cakes. The photomutagenic property of maltol, however, would not be serious in respect to human health risk, since a small amount of maltol is used in food products as a flavour enhancer. Rather we interested in the photomutagenic mechanism involved in maltol but not in kojic acid.

UV irradiation can generate photoproducts that cause mutations via different types of mechanisms. One is the generation of short-lived products such as reactive oxygen species or the molecules in their short-lived excited state that react directly with the DNA (3–7). Another is the formation of more stable photoproducts upon irradiation. Photomutagenicity of maltol was the latter case. Some aromatic and heterocyclic amines such as 2-aminofluorene, 2-acetylaminofluorene, 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-3,4-dimethylimidazo-[4,5-*f*]quinoline (MelQ) are reported to form highly stable direct-acting mutagenic photoproducts by irradiation with near-UV light (8). 7,12-Dimethylbenz[*a*]anthracene, a polycyclic aromatic hydrocarbon compound, showed direct-acting mutagenicity following fluorescent lamp illumination (9). Some *N*-nitroso compounds, such as *N*-nitrosopiperidine, *N*-nitrosodimethylamine and *N*-nitrosopyrrolidine, are another class of photomutagens that were converted to direct-acting mutagens by UVA-irradiation (10–13). However, the mechanisms leading to photomutagenicity as well as the identification of mutagenic photoproducts have not always been elucidated. Since the photomutagenic property of pyrone compounds has not been reported, we investigated the mechanisms of photoactivation of maltol in this study.

Materials and methods

Chemicals, bacterial strains and mutation test

Maltol (>99%) was purchased from Wako Pure Chemical Industries, (Tokyo) and dissolved in dimethyl sulfoxide (DMSO) at 100 mg/ml. L-cysteine and glutathione (reduced form) were purchased from Wako Pure Chemical Ind. and Sigma-Aldrich, MO, USA, respectively. We used *Salmonella typhimurium* strains TA100 [*hisG46, uvrB, rfa*/pKM101], TA98 [*hisD3052, uvrB, rfa*/pKM101], TA97 [*hisD6610, uvrB, rfa*/pKM101] and TA104 [*hisG428, uvrB, rfa*/pKM101] (14,15) for the reverse mutation assays and cultured them in Oxoid nutrient medium at 37°C. Minimal glucose agar plates consisted of Vogel–Bonner E medium supplemented with 2% glucose and 1.5% agar (14). Top agar (0.6% agar and 0.5% NaCl) contained 0.05 mM D-biotin and 0.05 mM L-histidine. We treated 0.1 ml bacterial culture in a test tube with the UVA (or UVC)-irradiated maltol solution (0.5 ml) at 37°C for 20 min. We plated the mixture with 2 ml molten top agar, incubated the plates for 2 days at 37°C and then counted the number of His^+ revertant colonies. In another experiment, we added 50 μl cysteine or glutathione solution (dissolved in phosphate buffer, pH 7.4) to the UVA-irradiated maltol solution and incubated it at 37°C for 5 min prior to exposure to bacterial cells. All experiments were conducted in duplicate, the control in triplicate. We judged as positive a doubling of the control value together with a dose-related response.

*To whom correspondence should be addressed. Tel: +81 3 3362 7420; Fax: +81 3 3362 8522; Email: akanuma@kureha.co.jp

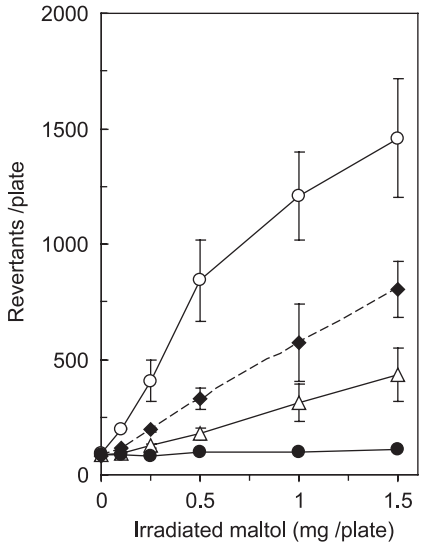

Fig. 1. Photomutagenicity of UV-irradiated maltol in *S. typhimurium* TA100. Maltol was irradiated in sodium phosphate buffer (100 mM, pH 7.4) with UVA for 5 (open triangle) or 30 min (open circle), or UVC for 3 min (filled diamond) prior to addition of bacterial cells. Unirradiated maltol (filled circle) showed no mutagenicity. Data are the mean of two experiments. The vertical bars indicate the range in the two experiments.

We used *Escherichia coli* strains WP3101P–WP3106P (16) to determine the mutational spectrum. These strains are F′ plasmid-carrying derivatives of strain WP2000P [*uvrA155, trpE65,* Δ*(lac-pro)*/pKM101] and exhibit a Lac^-, Trp^- phenotype. Each of the six F′ plasmids (*lac*$^-$, *lacZ*$^-$, *proAB*$^+$) has a unique *lacZ* mutation at the codon for Glu-461 (17). The mutations necessary for Lac^+ reversion are A:T→C:G for WP3101P, G:C→A:T for WP3102P, G:C→C:G for WP3103P, G:C→T:A for WP3104P, A:T→T:A for WP3105P and A:T→G:C for WP3106P. The MGT medium, ML agar plates, NBT-top agar and the assay method have been described previously (16,18). We conducted the experiments in triplicate for each dose and used five plates for the control. The data presented in the figures are the means of two experiments.

UVA and UVC irradiation

We added maltol (1–15 μl of 100 mg/ml solution dissolved in DMSO) into 0.5 ml 100 mM sodium phosphate buffer (pH 7.4) in each well of a 24-well multiplate and then irradiated it with UVA for 5–30 min or UVC for 1–3 min at room temperature. In some experiments, we used sodium phosphate buffer with a different pH, 10 mM Tris–HCl buffer, 100 mM NaCl or 10 mM $NaHCO_3$. We used a black-light fluorescent lamp (National Black Light Blue, FL15BL-B, 15W, Matsushita Electric Industrial, Japan) that emitted wavelengths of 300–400 nm as the UVA source. To filter out UVB wavelengths below 320 nm, a 5 mm thick soft glass plate was used (10–12,19). The solution in a lidded 24-well multiplate was irradiated with UVA from a distance of 20 cm at 230 μW/cm^2 (UVX Radiometer, Model UVX-36, Ultra-Violet Products, Upland, CA, USA). On the other hand, we used a germicidal lamp (Toshiba, GL-15) as the UVC source and irradiated solutions on a multiplate without a lid at 610 μW/cm^2 (UVX Radiometer, Model UVX-25).

8-OHdG Assay

We dissolved maltol in 10 mM Na-phosphate buffer at 10 mg/ml. We dissolved salmon sperm DNA (Wako Pure Chemical) in TE buffer at 5 mg/ml and dialysed it against 10 mM Na-phosphate buffer (pH 7.4). We mixed DNA (0.5 mg) and maltol (0.06, 0.13, 0.25, 0.5 and 1.0 mg) into 0.25 ml sodium phosphate buffer in each well of a 24-well multiplate (final concentrations of maltol were 0.25, 0.5, 1, 2 and 4 mg/ml), irradiated the mixture with UVA for 20 min, and then dialysed the reaction mixture against water. *N*-nitrosopyrrolidine (Sigma-Aldrich) dissolved in sodium phosphate buffer was used as a positive control (12, 20). We treated the dialysed DNA with Nuclease P1 (5 unit, Seikagaku, Tokyo) in sodium acetate buffer (20 mM, pH 4.8) at 37°C for 2 h, and then by bacterial alkaline phosphatase (1 unit, Takara Bio Inc., Shiga, Japan) in Tris–HCl buffer (100 mM, pH 8.0) at 37°C for an additional 2 h (20). We then removed the enzymes by ultrafiltration (14 000 *g*, 10 min) with Microcon YM-30 (Millipore, USA), added 1 μl 500 mM EDTA (pH 8.0) to the filtered samples and kept the mixture at −20°C. We measured the amount of deoxyguanosine (dG) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) using a high-performance liquid chromatography (HPLC) system (Tosoh, Japan). The system was composed of two pumps (CCPM-II), a degasser (SD-8020), an autosampler (AS-8020), a column oven (CO-8020), a reverse-phase column (Hyderosphere C18, 5 μm, 150 × 4.6 mm ID, YMC, Japan), a UV detector (UV-8020) and an electrochemical detector (ECD, EC-8020). We used Tosoh HPLC Multi Station software (LC-8020) for data acquisition. A 25 μl of sample was injected into the HPLC system. 8-OHdG and dG were eluted at a flow rate of 1 ml/min. We used two solvents for gradient elution—solvent A (2% acetonitorile in 10 mM phosphate buffer and 1 mM EDTA, pH 7.0) and solvent B (8% acetonitrile in the same buffer). The elution conditions were as follows: 0–5 min 0% solvent B; 5–20 min, 0–100% solvent B; 20–25 min 100% solvent B isocractic; 25–30 min 100–0% solvent B. The column temperature was maintained at 35°C. 8-OHdG was detected with the ECD equipped with glassy carbon working electrode operated at +500 mV versus a Ag/AgCl reference electrode. dG was measured by UV absorption at 254 nm. Data were expressed as 8-OHdG/10^5 dG (nM).

Table I Effect of pH on photoactivation of maltol

Solvent used for UVA or UVC-irradiation	His^+/plate (mean)	Induced His^+/plate*	Relative mutagenicity (%)
UVA-irradiated maltol (1 mg/plate)			
100 mM Na-phosphate (pH 7.4)	811	695	100
10 mM Na-phosphate (pH 7.4)	957	841	121.0
10 mM Na-phosphate (pH 5.5)	290	174	25.0
10 mM Na-phosphate (pH 6.0)	486	370	53.2
10 mM Na-phosphate (pH 6.5)	865	749	107.8
10 mM Na-phosphate (pH 7.0)	830	714	102.7
10 mM Na-phosphate (pH 8.0)	1013	897	129.1
10 mM Na-phosphate (pH 8.4)	993	877	126.2
10 mM Tris–HCl (pH 7.5)	746	630	90.6
10 mM Tris–HCl (pH 8.3)	1027	911	131.1
10 mM $NaHCO_3$ (pH 8.9)	897	781	112.4
100 mM NaCl (pH 6.0)	142	26	3.7
Water	131	15	2.2
UVC-irradiated maltol (1 mg/plate)			
10 mM Na-phosphate (pH 7.4)	800	684	100
10 mM $NaHCO_3$ (pH 8.9)	757	641	93.7
100 mM NaCl (pH 6.0)	804	688	100.6
Water	714	598	87.4
Control: unirradiated maltol (1 mg/plate)			
10 mM Na-phosphate (pH 5.5)	116	–	–
10 mM Na-phosphate (pH 7.4)	106	–	–
10 mM Na-phosphate (pH 8.4)	101	–	–
10 mM Tris–HCl (pH 8.3)	109	–	–
10 mM $NaHCO_3$ (pH 8.9)	114	–	–
100 mM NaCl (pH 6.0)	110	–	–
Control: untreated			
100 mM Na-phosphate (pH 7.4)	116 ± 10.3 (mean ± SD of 4 experiments)		

Ten microlitres of maltol (100 mg/ml) dissolved in DMSO was added to 0.5 ml of the indicated buffer or solution, and irradiated with UVA for 20 min or UVC for 3.5 min. Immediately after the irradiation, bacterial culture (0.1 ml) was added and pre-incubated at 37°C for 20 min before plating. Data are the mean of two experiments, each using duplicate plates.
*Subtracted the number of untreated control (116).

Results

Photomutagenic property

When maltol was irradiated with UVA for 5–30 min in 100 mM sodium phosphate buffer (pH 7.4) prior to incubation with bacterial cells (TA100), it was mutagenic in proportion to the irradiation time (Figure 1), while non-irradiated maltol was not mutagenic. UVC-irradiation also effectively activated maltol (Figure 1). At pH 7.4, 10 mM sodium phosphate was more conductive than 100 mM to photoactivation (Table I). Photoactivation was pH dependent. In 10 mM sodium phosphate buffer, photomutagenicity decreased with pH starting at pH 6.0. Photomutagenicity was observed in 10 mM Tris–HCl

buffer (pH 7.5 and 8.3) and 10 mM $NaHCO_3$ (pH 8.9) indicating that photoactivation did not require phosphate anion. UVA-irradiation did not activate maltol in 100 mM NaCl or in water (Table I). UVC, however, did activate maltol in 100 mM NaCl, suggesting photoactivation of maltol required greater energy in NaCl solution than in other buffer solutions.

Mutational specificity and 8-OHdG

When UVA-irradiated maltol was assayed with 4 different tester strains, it was mutagenic in TA100 (14 times the control value at 1 mg/plate), less in TA97 and TA104 (~5 times the control value at 1 mg/plate), and almost non-mutagenic to TA98 (Figure 2). Therefore, UVA-irradiated maltol induced both −1 frameshift and base-substitution mutations. To investigate the spectrum of the base-substitutions we conducted a Lac^+ reversion assay using *E. coli* strains WP3101P–WP3106P. UVA-irradiated maltol induced predominantly G:C→T:A transversions (15 times the control value) and G:C→A:T transitions (9 times the control) as shown in Figure 3. Other types of base pair substitutions were not detected. Since G:C→T:A transversions, which are typically caused by oxidative DNA damage (21), predominated, we investigated the contribution of 8-hydroxyguanine to the photomutagenicity of maltol. The ratio of 8-OHdG/dG in a UVA-irradiated mixture of DNA and maltol in an *in vitro* experiment was ~1.7 times that of the corresponding non-irradiated control (Figure 4). The results indicated that the contribution of 8-OHdG to the photomutagenicity of maltol was very small.

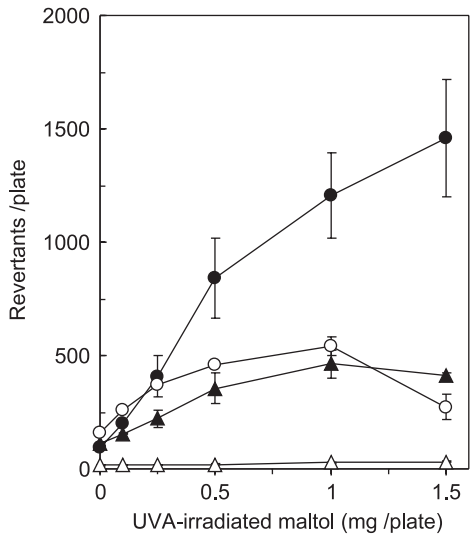

Fig. 2. Photomutagenicity of UVA-irradiated maltol in *S. typhimurium* strains. Maltol was irradiated in sodium phosphate buffer (100 mM, pH 7.4) with UVA for 30 min and then assayed with TA100 (filled circle), TA104 (open circle), TA97 (filled triangle) and TA98 (open triangle). Data are the mean of two experiments. The vertical bars indicate the range in the two experiments.

Stability of photoactivated maltol and inactivation by cysteine

The photomutagenic product(s) derived from UVA-irradiated maltol was considerably stable in a pH 7.4 sodium phosphate buffer. Maltol solution kept at room temperature in the dark after UVA-irradiation was equally mutagenic to TA100 after 30 min, and about 80% mutagenic activity remained at 60 min (Figure 5). Similar results were obtained in TA97 (data not shown). On the other hand, the addition of thiol compounds diminished the mutagenicity. At 200 μg cysteine/well (dissolved in sodium phosphate buffer, pH 7.4), the number of induced revertants of TA100 was reduced by 80% (Figure 6). Similar results were observed with glutathione (data not shown).

Discussion

We previously reported that thiabendazole (TBZ), a post-harvest fungicide, shows potent mutagenicity following UVA irradiation, probably by adduct formation, in *E. coli* WP2*uvrA*/pKM101 and the predominant mutations were G:C→A:T transitions and A:T→T:A transversions (7). The photomutagenic product was unstable, because photomutagenicity was observed when cells were irradiated by UVA in the presence of TBZ, but pre-irradiated TBZ was not mutagenic (22). Unlike the TBZ photoproducts, the maltol photoproducts were stable and had mutagenic activity at least 60 min after irradiation. The product, however, were relatively unstable (or less formed) under acidic conditions compared with neutral and alkaline

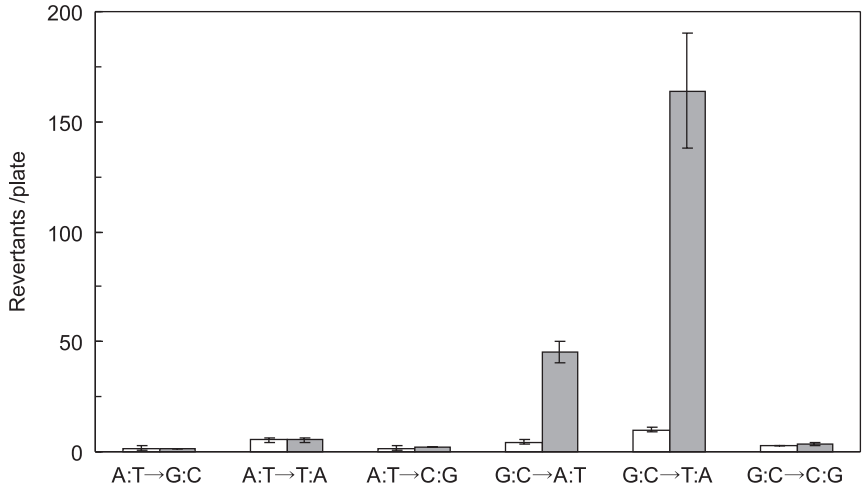

Fig. 3. Base substitutions induced by UVA-irradiated maltol in *E. coli* strains WP3101P-WP3106P. Maltol was irradiated in sodium phosphate buffer (100 mM, pH 7.4) with UVA for 30 min. Open bar, spontaneous Lac^+ revertants/plate; grey bar, Lac^+ revertants/plate induced by UVA-irradiated maltol at 1 mg/well. Data are the mean of two experiments. The vertical bars indicate the range in the two experiments.

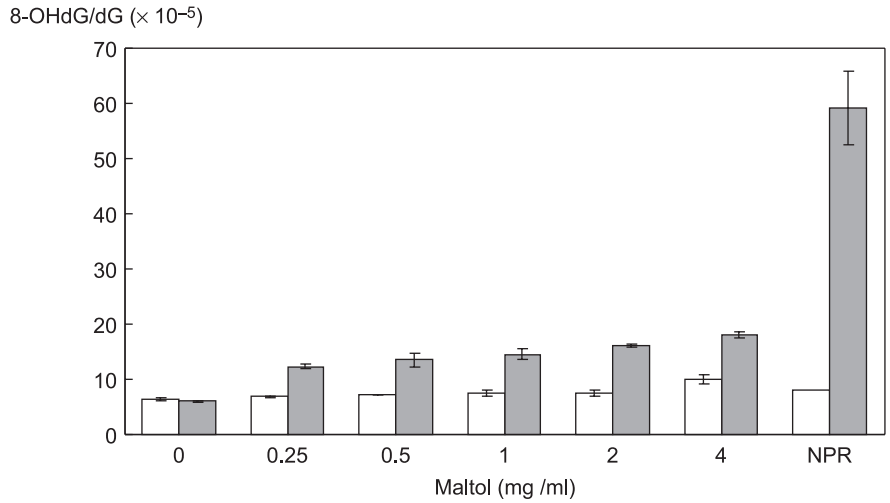

Fig. 4. 8-OHdG formation in DNA exposed to maltol and UVA. A mixture of DNA and maltol (0, 0.25, 0.5, 1, 2 and 4 mg/ml) in sodium phosphate buffer (10 mM, pH 7.4) was irradiated with UVA for 30 min. NPR (2 mg/ml) was used as a positive control. Open bar, DNA and maltol (or NPR) without UVA; grey bar, DNA and maltol (or NPR) with UVA. Data are the mean of two experiments. The vertical bars indicate the range in the two experiments.

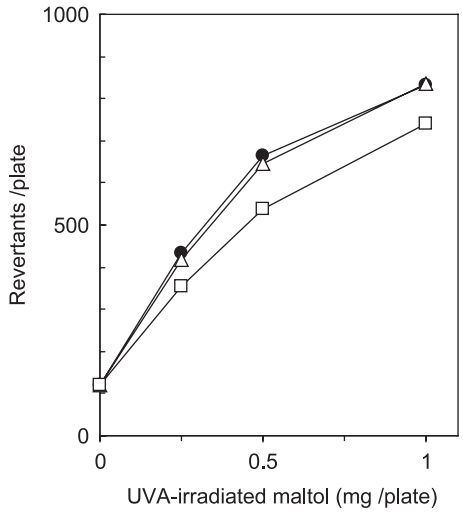

Fig. 5. Stability of mutagenic products in UVA-irradiated maltol. Maltol at indicated doses was irradiated in sodium phosphate buffer (100 mM, pH 7.4) with UVA for 10, 20 or 30 min. Immediately after (filled circle), or 30 (open triangle) and 60 min (open square) after the irradiation, mutagenicity was assayed with TA100. Representative data obtained from UVA-irradiation for 20 min was presented.

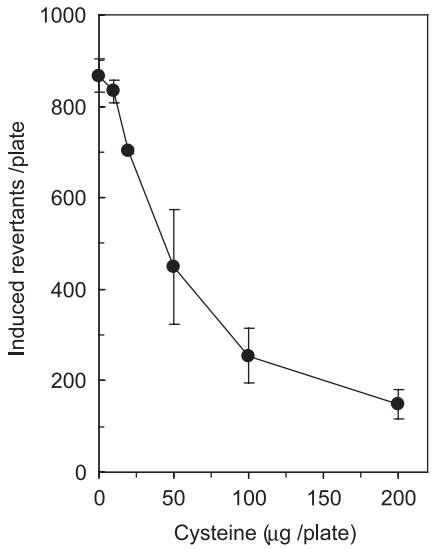

Fig. 6. Inhibitory effect of cysteine on photomutagenicity of maltol. Maltol at 1 mg/well was irradiated in sodium phosphate buffer (100 mM, pH 7.4) with UVA for 20 min. Cysteine (dissolved in the same buffer) was added and the mixture was kept at room temperature for 5 min prior to addition to bacteria. Data are the mean of two experiments. The vertical bars indicate the range in the two experiments.

conditions (Table I) and quickly reacted with thiol compounds, resulting in loss of mutagenicity (Figure 6). The formation of mutagenic phosphate esters by UVA-irradiated *N*-nitrosopyperidine and *N*-nitrosopyrrolidine has been reported (10,23), but that does not appear to be a mechanism involved with maltol, however, because photomutagenicity was observed with UVA-irradiation in Tris–HCl buffer and $NaHCO_3$ solution (Table I). Analysis of the mutagenic specificity of UVA-irradiated maltol revealed that the predominant base-substitutions induced were G:C→T:A transversions, but formation of 8-OHdG was not likely to be an important lesion. In addition, −1 frameshift mutations (TA97) were efficiently induced. Photomutagenicity of maltol was not detected in excision repair-proficient (*uvrB*$^+$) strains TA102 and TA92 (data not shown), suggesting that the DNA damage generated was repaired by the excision repair system. Although the photomutagenic mechanism of UVA-irradiated maltol has not been clarified, we suspect that DNA bulky adduct formation may be involved.

It has been reported that 5-hyroxymaltol, a glucose pyrolysate, is almost as mutagenic to TA100 as to *E. coli* WP2*uvrA*/pKM101 (24). UVA-irradiated maltol, on the other hand, was barely mutagenic to WP2*uvrA*/pKM101 (data not shown). We are trying to isolate the mutagenic compound by HPLC. In our preliminary analysis of an active HPLC fraction by LC-TOF/MS, we could not find a signal corresponding to 5-hydroxymaltol (*m*/*z* 142) among several signals detected (M. Watanabe-Akanuma, unpublished data). Further investigation is needed to identify the mutagenic compound(s) and clarify the photomutagenic mechanism.

On the other hand, there were contradictory reports on the bacterial mutagenicity of maltol itself. It was reported to be a weak mutagen to *S. typhimurium* TA100 (25), but others obtained negative results in the Ames/*Salmonella* test with and without S9mix (26). In our present assay, maltol without UV-irradiation was not mutagenic to *Salmonella* strains TA100, TA98, TA97 and TA104. Mutagenic photoproduct of maltol might explain the discrepancy.

Acknowledgements

We thank Dr Miriam Bloom for her critical reading of the manuscript. This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan (18602003).

References

1. Nohynek,G.J., Kirkland,D., Marzin,D., Toutain,H., Leclerc-Ribaud,C. and Jinnai,H. (2004) An assessment of the genotoxicity and human health risk of topical use of kojic acid [5-hydroxy-2-(hydroxymethyl)-4H-pyran-4-one]. *Food Chem. Toxicol*., **42**, 93–105.
2. Ishikawa,S., Sasaki,Y.F., Kawaguchi,S., Mochizuki,M. and Nagao,M. (2006) Characterization of genotoxicity of kojic acid by mutagenicity in Salmonella and micronucleus induction in rodent liver. *Genes Environ*., **28**, 31–37.
3. Jose,J.G. (1979) Photomutagenesis by chlorinated phenothiazine tranquilizers. *Proc. Natl Acad. Sci. USA*, **76**, 469–472.
4. Oppenländer,T. (1988) A comprehensive photochemical and photophysical assay exploring the photoreactivity of drugs. *Chimia*, **42**, 331–342.
5. Ciulla,T.A., Epling,G.A. and Kochevar,I.E. (1986) Photoaddition of chlorpromazine to guanosine-5′-monophosphate. *Photochem. Photobiol*., **43**, 607–613.
6. Gocke,E. (1996) Review of the genotoxic properties of chlorpromazine and related phenothiazines. *Mutat. Res*., **366**, 9–21.
7. Watanabe-Akanuma,M., Ohta,T. and Yamagata,H. (2003) Photomutagenicity of thiabendazole, a postharvest fungicide, in bacterial assays. *Environ. Mol. Mutagen*., **41**, 92–98.
8. De Flora,S., Camoriano,A., Izzotti,A., D'Agonist,F. and Bennicelli,C. (1989) Photoactivation of mutagens. *Carcinogenesis*, **10**, 1089–1097.
9. Takahashi,K., Asanoma,M., Miyabe,M. and Watanabe-Akanuma,M. (2001) A note on artificial induction of mutation upon testing 7,12-dimethylbenz[*a*]anthracene mutagenicity under fluorescent light in the absence of microsomal enzymes. *Environ. Mutagen Res*., **23**, 103–106.
10. Arimoto,S., Shimada,H., Ukawa,S., Mochizuki,M. and Hayatsu,H. (1989) Formation of directly mutagenic alpha-hydroxy-*N*-nitrosopiperidine phosphate ester by near-ultraviolet irradiation of *N*-nitrosopiperidine in phosphate buffer. *Biochem. Biophys. Res. Commun*., **162**, 1140–1146.
11. Arimoto-Kobayashi,S., Kaji,K., Sweetman,G.M. and Hayatsu,H. (1997) Mutation and formation of methyl- and hydroxylguanine adducts in DNA caused by *N*-nitrosodimethylamine and *N*-nitrosodiethylamine with UVA irradiation. *Carcinogenesis*, **18**, 2429–2433.
12. Arimoto-Kobayashi,S., Anma,N., Yoshinaga,Y., Douki,T., Cadet,J. and Hayatsu,H. (2000) Oxidative damage and induced mutations in m13mp2 phage DNA exposed to *N*-nitrosopyrrolidine with UVA radiation. *Mutagenesis*, **15**, 473–477.
13. Arimoto-Kobayashi,S., Ando,Y., Horai,Y., Okamoto,K., Hayatsu,H., Lowe,J.E. and Green,M.H. (2002) Mutation, DNA strand cleavage and nitric oxide formation caused by *N*-nitrosoproline with sunlight: a possible mechanism of UVA carcinogenicity. *Carcinogenesis*, **23**, 1537–1540.
14. Maron,D. and Ames,B.N. (1983) Revised methods for the *Salmonella* mutagenicity test. *Mutation. Res*., **113**, 173–215.
15. Levin,D.E., Marnett,L.J. and Ames,B.N. (1984) Spontaneous and mutagen-induced deletions: Mechanistic studies in Salmonella tester strain TA102. *Proc. Natl Acad. Sci. USA*, **81**, 4457–4461.
16. Ohta,T., Watanabe-Akanuma,M., Tokishita,S. and Yamagata,H. (1999) Mutation spectra of chemical mutagens determined by Lac^+ reversion assay with *Escherichia coli* WP3101P-WP3106P tester strains. *Mutat. Res*., **440**, 59–74.
17. Cupples,C.G. and Miller,J.H. (1989) A set of *lacZ* mutations in *Escherichia coli* that allow rapid detection of each of the six base substitutions. *Proc. Natl Acad. Sci. USA*, **86**, 5345–5349.
18. Ohta,T., Watanabe-Akanuma,M. and Yamagata,H. (2000) A comparison of mutation spectra detected by the *Escherichia coli* Lac^+ reversion assay and the *Salmonella typhimurium* His^+ reversion assay. *Mutagenesis*, **15**, 317–323.
19. Gocke,E., Muller,L., Guzzie,P.J., Brendler-Schwaab,S., Bulera,S., Chignell,C.F., Henderson,L.M., Jacobs,A., Murli,H., Snyder,R.D. and Tanaka,N. (2000) Considerations on photochemical genotoxicity: report of the International Workshop on Genotoxicity Test Procedures Working Group. *Environ. Mol. Mutagen*., **35**, 173–184.
20. Watanabe-Akanuma,M., Inaba,Y. and Ohta,T. (2006) Analysis of photomutagenicity of thiabendazole with UVA irradiation: absence of 8-hydroxyguanosine formation. *Genes Environ*., **28**, 103–107.
21. Cheng,K.C., Cahill,D.S., Kasai,H., Nishimura,S. and Loeb,L.A. (1992) 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G→T and A→C substitutions. *J. Biol. Chem*., **267**, 166–172.
22. Watanabe-Akanuma,M., Ohta,T. and Sasaki,Y.F. (2005) A novel genotoxic aspect of thiabendazole as a photomutagen in bacteria and cultured human cells. *Toxicol. Lett*., **158**, 213–219.
23. Arimoto-Kobayashi,S., Inada,N., Anma,N., Shimada,H. and Hayatsu,H. (1999) Induced mutations in M13mp2 phage DNA exposed to *N*-nitrosopyrrolidine with UVA irradiation. *Environ. Mol. Mutagen*., **34**, 24–29.
24. Kosuge,T., Tsuji,K., Nukaya,H., Terada,A., Ochiai,M., Wakabayashi,K., Nagao,M. and Sugimura,T. (1983) Isolation and identification of 5-hydroxymaltol, a mutagenic substance in glucose pyrolysate. *Agric. Biol. Chem*., **47**, 881–883.
25. Bjeldanes,L.F. and Chew,H. (1979) Mutagenicity of 1,1-dicarbonyl compounds: maltol, kojic acid, diacetyl and related substances. *Mutat. Res*., **67**, 367–371.
26. Ishidate,M.,Jr., Sofuni,T., Yoshikawa,K., Hayashi,M., Nohmi,T., Sawada,M. and Matsuoka,A. (1984) Primary mutagenicity screening of food additives currently used in Japan. *Food Chem. Toxicol*., **22**, 623–636.

Received on July 18, 2006; revised on September 12, 2006; accepted on October 26, 2006