The Mutagenic Activity of the Crude Extract Obtained from Silicon Dioxide and Titanium Dioxide with Pyrene and Sodium Chloride under Xenon Lamp Irradiation

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(Received October 18, 1999; Accepted January 6, 2000)

The mutagenic activity of the crude extract obtained from the reaction mixture of pyrene and sodium chloride in the presence of 2 metallic oxides [silicon dioxide: silicic anhydride form (SiO_2) and titanium dioxide: anatase form (TiO_2)] under xenon lamp irradiation was examined using *Salmonella typhimurium* tester strains (TA98 and TA100), and was compared with the mutagenic activity of 1-chloropyrene (1-CP), dichloropyrenes (DCP), 1-nitropyrene (1-NP) and dinitropyrenes (DNP) in the crude extract. In the presence of SiO_2, the mutagenicity of 1-NP in the crude extract, and that of the crude extract increased with the progress of the irradiation time. Moreover, the crude extract and 1-NP levels of mutagenicity were considerably similar in each exposure time. Accordingly, it was presumed that the increase of the mutagenic activity of the crude extract was caused by the increase of the yields of 1-NP in the crude extract. In the presence of TiO_2, the mutagenicity of 1-NP in the crude extract decreased for the extended irradiation time, but the mutagenicity of the crude extract increased in reverse. It was presumed that the increase of the mutagenic activity of the crude extract was due to increasing the yields of other strong mutagens other than 1-CP, DCP or 1-NP.

Key words —— chlorinated pyrene, 1-nitropyrene, xenon lamp irradiation, silicon dioxide, titanium dioxide, mutagenicity

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INTRODUCTION

Pyrene exists mainly in coal tar, and is often detected in the soil as well.[1] Pyrene does not show any mutagenicity in the Ames test, but 1-CP and DCP have moderate mutagenicity.[2] It was previously reported that chlorinated pyrenes were detected from 9 sorts of metallic oxides, which are soil components, were added to pyrene and chloride ion, and then irradiated with a xenon lamp.[3] During these experiments, it was observed that the color of the extracted solution changed from colorless to yellow in the presence of silicon dioxide [silicic anhydride form (SiO_2)] and from yellow to light yellow in the presence of titanium dioxide [anatase form (TiO_2)] with the progress of xenon lamp irradiation time. From the above observation, it was expected that nitro compounds as reaction products would be produced in the presence of metallic oxides. Nitrated pyrenes are strong mutagens.[4,5] There are many reports about tracing nitrated pyrenes in environmental air.[6,7] In this paper, in order to clarify the effect of soil components towards the production of mutagens, crude extracts were prepared by the reaction of pyrene and sodium chloride under xenon lamp irradiation with SiO_2 and TiO_2. We examined the mutagenicity of organic extracts in each irradiation time using Ames assay. We quantified 1-CP, DCP and 1-NP in the crude extract and estimated the contribution of the compounds to the mutagenicity of the extracts.

MATERIALS AND METHODS

Materials —— Pyrene was a standard reagent (99%) produced by GL-Science Ltd. 1-NP was purchased from Tokyo Kasei Co., Ltd. 1,3-, 1,6- And 1,8-DNP were purchased from Aldrich Co., Ltd. 1-CP and DCP were kindly provided by Dr. Y. Mori of Kanagawa Prefectural Public Health Laboratory.[8] SiO_2(silicic anhydride form, 99.9%) and TiO_2(anatase form, <5 μm, 99.9%) were purchased from Wako Pure Chemical Industries Co., Ltd. The other materials were described previously.[9]

Equipment —— Xenon lamp irradiation apparatus: Xenon long life weather meter WEL-45AX, Suga Test Instruments Co., Ltd. The spectrum of xenon lamp rises at 290 nm, and is practically similar with the spectrum of sunlight. The other equipment were

described previously.[9]

Xenon Lamp Irradiation —— Two g each of metallic oxide treated with sodium chloride (800 μg as chloride ion) and pyrene (500 μg) were put into each laboratory dish and irradiated with the xenon lamp for 0.5, 1, 3, 6 and 12 h, respectively. After irradiation by lamp, the reaction product was extracted with benzene: ethanol mixed solution 100 ml (4 : 1, v/v) and the solution was concentrated to 2 ml. 1-CP, DCP, 1-NP and DNP in the extracted solution were analyzed by gas chromatograph/mass spectrometer (GC/MS). The details of the determination methods were described previously.[10]

Mutagenicity Test —— The sample solution of 2 ml was filtered by Teflon filter and the solvent was completely volatilized in a nitrogen gas stream and this crude extract was weighed. Mutagenicity assays were performed by the Ames method[11] using *Salmonella typhimurium* TA98 and TA100 with and without S9 mix(+S9 and -S9), and were conducted in triplicate for each sample. The mutagenicity potency (rev./μg) of 1-CP, DCP and 1-NP used to calculate each mutagenicity of these compounds in extracts were as follows: 1-CP, 3 in TA98(+S9) and 6 in TA100 (+S9),[2] DCP, 2 in TA98(+S9) and 9 (+S9) in TA100,[2] respectively. The mutagenic potency of DCP was described as the mixed values of 1,3-, 1,6- and 1, 8-DCP.[8] In this paper, these values were used to the mutagenic potency of DCP. 1-NP, 230(+S9) and 1900 (−S9) in TA 98, and 142(+S9) and 482(−S9) in TA100,[4,5] respectively. Without S9 mix, chlorinated pyrenes did not show mutagenic potency to either strain.[2]

RESULTS AND DISCUSSION

The yields of 1-CP, DCP and 1-NP in the crude extract for the several irradiation times in SiO_2 and TiO_2 are shown in Table 1. The detection limits of 1-CP, DCP, 1-NP and DNP were 1 pg, 0.5 pg, 8 pg and 20 pg, respectively. DNPs were not detected in any samples.

The results of the mutagenicity assay of the crude extract obtained from the photochemical reaction in SiO_2 and TiO_2 are shown in Table 2 and Table 3, respectively. From these values and the amounts of the total extracts obtained from the extracted solution of 2 ml, the net revertants of the total extracts were calculated in each exposure time, and were expressed as the net revertants per total extracts. The yields of 1-CP, DCP and 1-NP in the crude extract are shown in Table 1. On the other hand, the mutagenicity potency of 1-CP, DCP and 1-NP are shown in the Mutagenicity test section. The total net revertants of 1-CP, DCP and 1-NP in the crude extract were calculated from these values, and were expressed as the net revertants per total extracts. They are shown in Figs. 1 and 2.

The mutagenicity of chlorinated pyrenes with S9 mix was very small as compared with that of 1-NP in both metallic oxides and both strains(data not shown). In the SiO_2, the effect of irradiation time on the mutagenic activity without S9 mix is shown in Fig. 1. The net revertants of 1-NP in the crude extract and that of the crude extract in TA98 and TA100 increased as the irradiation time elapsed. The mutagenic contribution ratio of 1-NP in the crude extract without S9 mix had almost no change from 70% in 0.5 h to 80% in 12 h in TA98, and from 55% in 0.5h to 90% in 12h in TA100, respectively. Moreover, with S9 mix, the crude extract and 1-NP levels of mutagenicity were considerably similar in each exposure time (data not shown). Therefore, in the

Table 1. The Yields of Chlorinated Pyrenes and 1-Nitropyrene in the Crude Extract Obtained from Silicon Dioxide and Titanium Dioxide

Irradiation time (h)	Silicon dioxide			Titanium dioxide		
	1-CP	DCP($\times10^{-2}$) (μg/total extracts)	1-NP	1-CP	DCP($\times10^{-2}$) (μg/total extracts)	1-NP
0	0	0	0	0	0	0
0.5	1.22 ±0.092	0.5 ±0.057	1.4 ±0.17	26.6 ±2.9	69.4 ±3.4	0.372 ±0.034
1	1.0 ±0.055	0.4 ±0.028	1.5 ±0.12	25.8 ±0.77	92 ±0.91	0.288 ±0.02
3	1.4 ±0.19	0.5 ±0.055	8.46 ±0.076	12.8 ±0.64	42.6 ±4.7	0.164 ±0.018
6	2.0 ±0.16	0.7 ±0.063	12.7 ±1.1	8.34 ±0.75	23 ±1.4	0.074 ±0.0044
12	2.9 ±0.18	1.6 ±0.21	48.8 ±5.9	4.22 ±0.21	12 ±0.84	0.064 ±0.005

Pyrene: 500 μg, chloride ion: 800 μg, metallic oxide: 2 g. Data are shown as the mean±S.D. of triplicates.

Table 2. The Mutagenic Activity of the Crude Extract on Silicon Dioxide (Silicic Anhydride Form)

Irradiation time (h)	Dose (μg/plate)	Revertants/plate(mean±S.D.)			
		TA98		TA100	
		−S9	+S9	−S9	+S9
Control	0	27± 21	44± 3	44± 3	120± 0.5
0.5	8.88	265± 3.5	116± 16	186± 4	195± 15
	17.8	383± 38	221± 13	246± 8	230± 14
	35.5	629± 89	282± 0.5	310± 2.5	328± 1.5
1	3.63	183± 21	68± 1.5	159± 8.5	181± 0.5
	7.3	255± 11	111± 3	189± 18	235± 20
	14.5	423± 32	164± 6	270± 12	272± 20
3	8.6	640± 34	199± 18	658± 24	271± 57
	17.3	1140± 78	407± 0	1032± 81	423± 7.5
	35	1930±170	831± 13	1780± 29	722± 9
6	10	1060± 8	394± 17	591±105	492± 17
	20	1800±137	662± 75	743± 78	634± 58
	40	2820± 76	1168± 57	1151±106	886± 29
12	2.53	690± 30	106± 3	217± 18	250± 9
	5.06	900± 4	210± 2	301± 18	346± 1
	10.13	1990±290	344± 39	627± 50	477± 10

Table 3. The Mutagenic Activity of the Crude Extract on Titanium Dioxide(Anatase Form)

Irradiation time (h)	Dose (μg/plate)	Revertants/plate(mean±S.D.)			
		TA98		TA100	
		−S9	+S9	−S9	+S9
Control	0	27± 21	44± 3	44± 3	120± 0.5
0.5	8	85± 10	64± 7	133± 1	195± 0
	16	126± 1.5	84± 2	143± 7	203± 2
	32	145± 14	109± 7	170± 1.5	213± 12
1	7	97± 7	68± 0.5	136± 1.5	184± 6.5
	14	147± 7.5	99± 5	138± 14	210± 6
	28	195± 3.5	152±18	192± 15	250± 31
3	3.75	261± 20	70± 1	190± 21	208± 5.5
	7.5	415± 59	136± 3	213± 8.5	295± 7
	15	659± 38	300± 2.5	332± 3.5	493± 22
6	0.625	896± 37	178± 4	745± 75	311± 8
	1.25	2171± 11	316±10	809± 26	398± 26
	2.5	3300±336	782± 7	1393± 43	823± 22
12	23.1	1330±130	166±11	841±195	280± 4
	46.3	3070± 10	234±32	979± 46	310± 12
	92.5	5340±740	622± 6	1547± 7	634±190

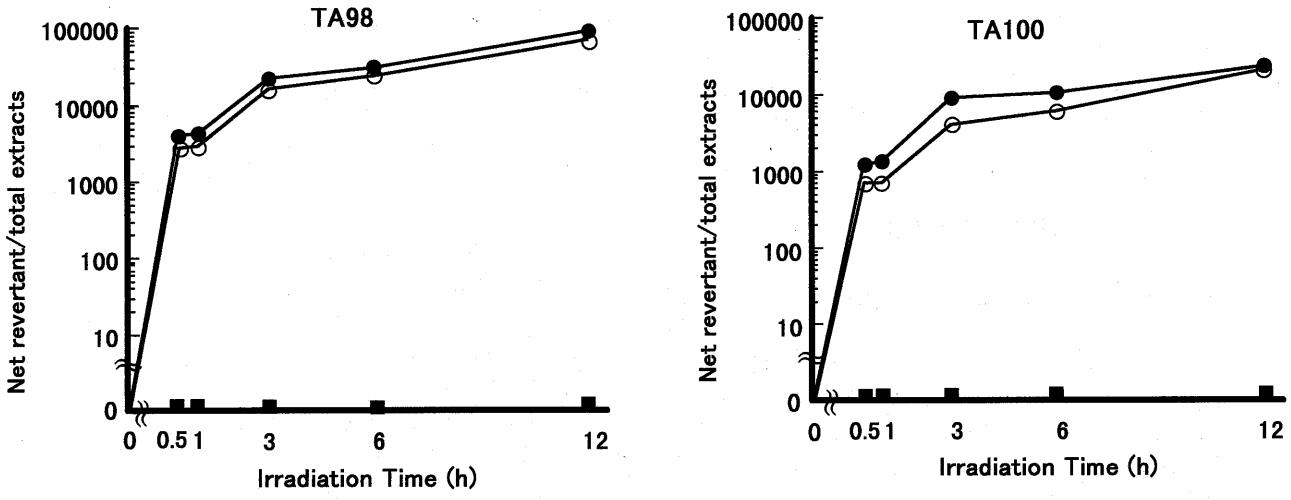

Fig. 1. The Mutagenicity of the Crude Extract, 1-CP, DCP and 1-NP Obtained from the Photo-chemical Reaction of Pyrene and Chloride Ion on Silicon Dioxide in TA98 and TA100 without S9 Mix

Mutagenic potency of 1-CP, DCP and 1-NP were calculated from their amounts (μg) in Table 1 and their revertants perμg described in experimental section (Mutagenicity test). Pyrene: 500 μg. Chloride ion: 800 μg. Silicon dioxide; 2 g. ●, Crude extract; ■, 1-CP; ▲, DCP; ○, 1-NP.

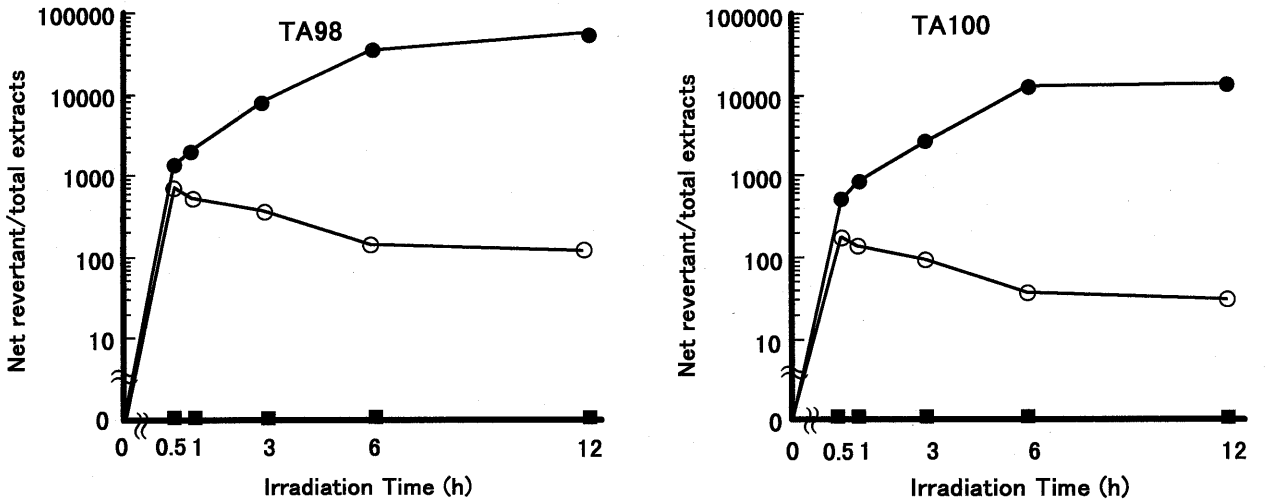

Fig. 2. The Mutagenicity of the Crude Extract, 1-CP, DCP and 1-NP Obtained from the Photo-chemical Reaction of Pyrene and Chloride Ion on Titanium Dioxide in TA98 and TA100 without S9 Mix

Mutagenic potency of 1-CP, DCP and 1-NP were calculated from their amounts (μg) in Table 1 and their revertants perμg described in experimental section (Mutagenicity test). Pyrene, 500 μg. Chloride ion, 800 μg. Titanium dioxide; 2 g. ●, Crude extract; ■, 1-CP; ▲, DCP; ○, 1-NP.

SiO_2, it was presumed that the increase of the mutagenic activity of the crude extract was caused by the increase of the yields of 1-NP in the crude extract as the irradiation time elapsed. In the TiO_2, the effect of irradiation time on the mutagenic activity of crude extract, 1-CP, DCP and 1-NP in TA98 and TA100 without S9 mix is shown in Fig. 2. The net revertants of 1-NP in the crude extract gradually decreased, but the net revertants of the crude extract were gradually increased as the irradiation time elapsed. The mutagenic contribution ratio of 1-NP in the crude extract decreased from 50% in 0.5 h to 0.2% in 12 h in TA 98, and decreased from 35% in 0.5 h to 0.22% in 12 h in TA100, respectively. Moreover, with S9 mix, a similar tendency was observed in each exposure time (data not shown). Accordingly, in the TiO_2, it was presumed that the increase of the mutagenic activity of the crude extract was caused by the increase of the yields of other strong mutagens other than 1-CP, DCP or 1-NP as the irradiation time was extended. From the

results described above, the mutational specificity produced by the photochemical reactions when exposed to silicon dioxide and titanium dioxide are expected to be different.

REFERENCES

1) Eckel W.P., Jacob T.A., *US DOE Rep.*, 81—88 (1992).
2) Mori Y., Naito S., Goto S., Matsushita H., *Jpn. J. Toxicol. Environ. Health*, **33**, 313—320 (1987).
3) Sugiyama H., Katagiri Y., Kaneko M., Watanabe T., Hirayama T., *Chemosphere*, **38**, 1937—1945 (1999).
4) Tokiwa H., Nakagawa R., Ohnishi Y., *Mutat. Res.*, **91**, 321—325 (1981).
5) Tokiwa H., *J.Japan Soc. Air Pollut.*, **27**, 73—84 (1992).
6) Randahl T., Becher G., *Environ. Sci. Technol.*, **16**, 861—865 (1982).
7) Ciccioli P., Cecinato A., Brancaleoni E., Draisci R., Liberti A., *Aerosol Sci. Technol.*, **10**, 296—310 (1989).
8) Mori Y., Goto S., Onodera Y., Naito S., Takitani S., Matsushita H., *Jpn. J. Toxicol. Environ. Health*, **38**, 282—287 (1992).
9) Sugiyama H., *Jpn. J. Toxicol. Environ. Health*, **42**, 38—43 (1996).
10) Sugiyama *H., Jpn. J. Toxicol. Environ. Health*, **42**, 92—95 (1996).
11) Ames B.N., McCan J., Yamasaki E., *Mutat. Res.*, **31**, 347—364 (1975).