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Studies on Mutagenicity of Ag-Os, a Water Treatment Agent

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ABSTRACT : **In order to evaluate the mutagenic potential of Ag-Os produced by receiving Ag ion at the carrier, 2 types of mutagenecity tests were performed. No mutagenic potential was shown in bacterial reverse mutation test using *Salmonella typhimurim* TA 1535, TA 1537, TA 98, TA 100. No DNA-damaging property was shown in Rec-assay using *Bacillus subtilis*(Rec+) and *Bacillus subtilis* (Rec-). These results indicate that the Ag-Os does not cause reverse mutation and DNA-damaging property**

Key word : **Ag-Os, reverse mutation, DNA-damage**

Introduction

Antimicrobial agent, organic mercury, organic chrominate and organic cupric compound had been used for hygiene. Antimicrobial action of these organic agents were excellent, but their usage were inhibited for the reason that they were reported that had mutagenicity (Tomioka *et al.* 1993). For the necessity of developing safe inorganic antimicrobial compound, silicate and zeolite were used as a inorganic carrier. As a antimicrobial metal, silver, copper and zinc can be exchanged in inorganic carrier. Since silver has high bonding energy with oxygen and same electric charge with Na^+, silver can make safest compound among these metals(Shin *et al.* 1997). As a antimicrobial agent, silver has been used for a long time, for example silvernitrate has been used for bacteriostatic and bacteriocidal agent and silvercitrate has been used for eliminating *Pseudomonas aeruginosa* (Eiji, 1991). Ag-Os that newly made water treatment agent was prepared using Oester shell as a inorganic carrier and exchanging siliver ion with Na+ at the carrier (Yamamoto *et al.*, 1991). Mutagenicities of Ag-Os were investigated in two types methodology, Ames test was done to find back mutation and the other Rec-assay was used to clarify the DNA-damaging property.

Materials and Methods

Material

Ag-Os (Shin *et al.*, 1995) was prepared by Environmental Engineering lab. in Dongseo University co-oprating with Myungkwang Chemical Industry. It has diameter 14.81 um, density 2.2 g/cm^3, purity 40.7 NTU at 0.1% sol., pH 9.7 at 0.1% sol. and surfactant area 20.0 m^2/g. This test material was used after dissolving in autoclaved distilled water.

Ames mutagenicity test

Mutagens/Carcinogens

2-Aminoanthracene (2-AA), 9-aminoacridine (9-AA), sodium azide (NaN_3) and 2-nitrofluorene (2-NF) were used as positive control. Those reagents were purchased from Sigma Chemical Co., St. Louis, Mo, USA, and weighed the appropriate amount and dissolved in DMSO or autoclaved distilled water.

Bacterial strans

Salmonella thyphimurium TA 1535, TA1537, TA 98, TA100 strain, histidine requiring mutant, were kindly provided by Dr. B.N. Ames, Univ. of Califonia, Berkeley, CA, USA and were maintained as described by Maron and Ames (1983). The genotype of the tester strain was checked routinely for the histidine requirement, deep rough(rfa) character, UV sensitivity (uvr B mutation) and the presence of R factor.

S9 fraction and S9 mix

Sprague-Dawley male rats were injected intraperitioneally

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with Aroclor 1254 dissolved in corn oil (500 mg/kg of body wt.). Five days after the injections, the rats were sacrified, the livers were removed and minced in 0.15M KCl, and then homogenized were centrifuged at 9000×g for 10 min in a refrigerated centrifuge and the supernatant S9 fraction was distributed in −80°C until used for mutagenic studies. In order to prepare the S9 mix, S9 fraction was thawed immediately before being used for the preparation of S9 mix. Ten percent of S9 fraction in S9 mix was used as S9 mix for the experiment.

Mutagenicity test

A modified plate incorporation test (Matsushima *et al.*, 1980) in which 30 min liquid preincubation of the organism with the test compounds was employed. In the preincubation test, 0.5 ml of S9 mix (or 0.5 ml phosphate buffer for direct mutagen) distributed in sterile capped tubes in ice bath and then 0.1 ml of testers from overnight culture ($1\sim2\times10^9$ cells/ml), 0.1 ml of test compound was added. The tubes were vortexed 3 seconds. The resulting entire mixture was overlaid on the minimal agar plate. The plate were incubated at 37°C for 48 hrs and then the revertants bacterial colonies on each plate were counted. Dose response tests on the mutagens on the tester strain were carried out to determine the regions of revealing mutagenicity induced by test compound. 0.1 ml of autoclaved distilled water was used in control plate.

Rec-assay

Mutagens/Carcinogens

4-nitro-guinoline-1-oxide (4-NQO) were used as positive control for showing clear inhibition zone. 4-NQO was obtained from Aldrich CO., Milwaukee, WI, U.S.A and dissolved in 95% ethanol.

Bacterial strain

Bacillus subtilis H-17 (Rec^+) and *Bacillus subtilis M-45* (Rec^-) strains were provided by Gene Bank of Life Engineering Center in KIST.

Assay

Methodology was done by a little modification of Kuboyam's (1986). Rec^+ and Rec^-, each strains were streaked seperately from one starting point in B-2 agar plate refraining from being mixed of two strains in one starting point. Test compound was dissolved in autoclaved distilled water, then the disc (12 mm) abosorbed with test compound was put at one starting point of two strains. Plates were incubated at 37°C for 24 hrs and distilled water was used as negative control and 4-NQO was used as positive control. DNA repair of test compound is deceided when the value of inhibition region of Rec- minus that of Rec+ is more than 2.0 mm.

Table 1. Reverse mutation test of Ag-Os in *Salmonella typhymurium*

compound	Dose (ug/plate)	S-9 mix	No of revertants per plate (Mean±SD)			
			TA1535	TA1537	TA98	TA100
Ag-Os	10,000	−	30±6	21.0±4	16±4	31±1
	5,000	−	33±5	23±4	25±5	19±4
	2,500	−	23±7	18±2	10±3	13±4
	1,250	−	19±4	17±3	11±3	16±3
	625	−	28±6	15±4	17±3	18±6
Control	0	−	25±2	14±2	12±4	19±4
NaN_3	0.5	−	371±42			230±17
9-AA	50	−		1400±216		
2-NF	1	−			624±84	
Ag-Os	10,000	+	16±4	21±6	10±7	42±3
	5,000	+	16±4	19±4	12±6	46±4
	2,500	+	12±6	16±5	6±4	42±11
	1,250	+	7±4	11±6	17±6	37±3
	625	+	9±1	12±3	10±5	38±7
Control	0	+	10±4	14±2	9±3	42±4
2-AA	0.5	+			73±5	
	1	+				169±56
	2	+	89±9	104±17		

9-AA: 9-aminoaridine·HCl. 2-NF: nitrofluorene. 2-AA: 2-aminoanthracene. n=3

Table 2. Rec-assay of Ag-Os, water treatment agent

compound	concentration (mg/disc)	Length of inhibition zone[a] M-45 (Rec^-)	H-17 (Rec^+)	Inhibition zone (Rec^--Rec^+) mm
Ag-Os	10,000	0.0±0.0	0.0±0.0	0.0±0.0
	5,000	0.0±0.0	0.0±0.0	0.0±0.0
	2,500	0.1±0.1	0.0±0.0	0.0±0.0
	1,250	0.2±0.1	0.0±0.0	0.0±0.0
	625	0.0±0.0	0.0±0.0	0.0±0.0
Control	0	0.0±0.0	0.0±0.0	0.0±0.0
4-NQO	200	23.6±3.2	0.0±0.0	23.6±3.2*

[a]Mean±SD for 3 plates
*More than 2 mm of inhibition zone

Statistical analysis

Statistical analysis was performed by analysis of variance. Significant differences between treatment means were determined by using the Student's t test.

Result and Discussion

In a reverse mutation test using Salmonella typhimurium TA 1535, TA 1537, TA 98, TA 100, Ag-Os did not show significant increase in reverse mutation colony at all doses of Ag-Os (Table 1). But positive control materials showed significant increases in mutation colony compared to control. In Rec-assay, larger value than 2 mm of a growth inhibition zone was not observed in all doses of Ag-Os (Table 2). But positive control material showed larger than 2 mm of a growth inhibition zone compared to control. All doses of Ag-Os did not show DNA-damaging property. In those two experiments, maximal concentration, 10 mg/plate is 2,000 fold the expected usage concentration for antimicrobial action. In conclusion, Ag-Os, a water treatment agent is considered very safe from the result that did not show reverse mutation and DNA-damaging property.

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수질 정화제로 개발한 Ag-Os의 변이원성 시험

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굴(**Oester**)껍질에 은 이온을 도입해 개발한 수질 정화제 **Ag-Os**의 복귀 돌연변이원성을 관찰하기 위하여, ***Salmonella typhymurium*** **TA 1535, TA 1537, TA 98, TA 100** 이용하였고, **DNA**-손상여부를 관찰하기 위하여 ***Bacillus Subtils*** **H-17(Rec$^+$)**와 **H-45(Rec$^-$)**을 이용하였다. **Ag-Os**에 의한 복귀 돌연변이는 관찰되지 않았고, 이는 **S 9**을 첨가시에도 같은 현상을 나타내었다. **Rec-assay**에 의한 **DNA** 손상도 관찰되지 않은 결과로 미루어, 수질 정화제 **Ag-Os**는 본 시험 조건에서 변이원성을 보이지 않음을 확인하였다.