Bull. Environ. Contam. Toxicol. (1987) 39:843–847

Bulletin of Environmental Contamination and Toxicology

Mutagenicity of Nitrohumic Acid in *Salmonella typhimurium* Strains

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Humic materials constitute the major organic constituents of soil. Humic acid generally is the material extracted from soil by alkaline solutions and is precipitated upon acidification. Humic acid itself does not demonstrate mutagenic activity. However, it has been shown recently that direct-acting mutagenic activity is formed following chlorination of humic materials (Bull et al. 1982; Meier et al. 1983).

The products obtained by treatment of lignites and brown coals with nitric acid have been used as a soil amendment especially in Japan (Sakai 1975; Fujinami 1977; Kruglov et al. 1979). The products are named the nitrohumic acid and are commercially available. Many nitrocompounds have been reported to be mutagenic in Salmonella typhimurium strains (Chiu et al. 1978; Tokiwa et al. 1981). However, the mutagenicity of nitrated humic materials remains to be studied.

In the present report, we have investigated whether the nitrohumic acid is mutagenic or not in Salmonella typhimurium strains.

MATERIALS AND METHODS

The humic acid (obtained from Aldrich) and the nitrohumic acid (obtained from Tokyo Kasei) were dissolved in 0.1 M sodium hydroxide solution. After centrifugation, the humic acid and the nitrohumic acid were precipitated by acidification of the supernatant solution to pH 1.0. Another sample of nitrohumic acid was prepared in our laboratory by heating humic acid (10 g, Aldrich) in 20% nitric acid solution (75 ml) at 80°C for 1 hr. This sample was precipitated by acidification of the alkaline supernatant solution in the same way as for the commercial samples of humic acid and nitrohumic acid. These precipitated samples were washed with neutral water, dried in a desiccator under reduced pressure and used for the elementary analysis and the mutagenicity test.

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Table 1. Mutagenicity of the nitrohumic acid in Salmonella typhimurium strains TA100 and TA98. Each value is the mean ± SD of 4-6 plates. Significantly different from each control (0 μg/plate, spontaneous) at *P < 0.05, **P < 0.01, ***P < 0.001 (the Student's t-test).

Amount of sample (μg/plate)	Mutagenicity (revertants/plate)		
	HA	NHA	PNHA
S. typhimurium TA100			
0 (spontaneous)	147 ± 3.0	166 ± 4.8	147 ± 3.0
10	153 ± 5.6	180 ± 5.8	212 ± 5.0*
100	144 ± 4.6	231 ± 8.9*	557 ± 14.7***
1000	147 ± 4.3	372 ± 11.3**	1114 ± 16.4***
S. typhimurium TA98			
0 (spontaneous)	34 ± 3.8	37 ± 4.8	34 ± 3.8
10	33 ± 5.0	57 ± 7.4	93 ± 5.3**
100	31 ± 2.2	132 ± 6.8***	553 ± 12.3***
1000	35 ± 5.6	260 ± 14.8***	

Anal. Found; the humic acid (Aldrich, HA): C, 51.95; H, 4.42; N, 0.69%; the nitrohumic acid (Tokyo Kasei, NHA): C, 52.23; H, 3.67; N, 3.72%; the nitrohumic acid prepared in our laboratory (PNHA): C, 50.97; H, 4.93; N, 2.52%.

Bacterial strains used in the experiment are Salmonella typhimurium TA100, TA98, TA102 and TA96. The mutagenicity test was performed as follows. The liquid preincubation method (Yahagi et al. 1977), a modified method of the test described by Ames et al. (1975) was used for TA100 and TA98. The same method was also used for TA102 and TA96, without using Oxoid nutrient broth No.2 in which tester strain cultures were grown for 12 hrs. Many mutagenic nitrocompounds are direct-acting mutagens which do not require metabolic activation by S9 mixtures and in the present report the mutagenicity test was performed without adding S9 mixtures (containing microsomes).

RESULTS AND DISCUSSION

HA did not show significant mutagenicity in the range of 10-1000 μg/plate in S. typhimurium strains TA100 and TA98

Table 2. Mutagenicity of the nitrohumic acid in Salmonella typhimurium strains TA102 and TA96. Each value is the mean ± SD of 3-6 plates. Significantly different from each control (0 μg/plate, spontaneous) at *P < 0.05, **P < 0.01, ***P < 0.001 (the Student's t-test).

Amount of sample (μg/plate)	Mutagenicity (revertants/plate)		
	HA	NHA	PNHA
S. typhimurium TA102			
0 (spontaneous)	292 ± 10.0	285 ± 6.4	292 ± 10.0
10	285 ± 7.7	302 ± 5.0	321 ± 10.6
100	281 ± 12.6	331 ± 10.2	396 ± 6.3
1000	279 ± 8.0	394 ± 13.4*	587 ± 15.8*
S. typhimurium TA96			
0 (spontaneous)	97 ± 6.9	101 ± 6.5	97 ± 6.9
10	102 ± 6.1	114 ± 8.8	134 ± 5.5**
100	105 ± 8.3	153 ± 7.1**	288 ± 11.3***
1000	103 ± 11.9		

which have guanine-cytosine base pairs at the critical site for reversion, while NHA increased the mutagenic activity with the increase of its concentration in the same strains (Table 1). PNHA was also mutagenic in S. typhimurium strains TA100 and TA98. Rosenkranz and Mermelstein (1983) have reported that the nitroarenes are potent frameshift mutagens. Many mutagenic nitrocompounds appear to be more responsive to TA98 (a frameshift mutation strain) than to TA100 (a base-pair substitution mutation strain). NHA and PNHA were more responsive to TA98 than to TA100 (Table 1). Therefore, the nitrohumic acid appears to have responsiveness similar to other nitrocompounds. The percentage of nitrogen in HA was 0.69% from the analytical result, while that in NHA was 3.72%. The percentage of nitrogen in the nitrated humic acid prepared in our laboratory was 2.52%. These results may show that the mutagenicity of the nitrohumic acid mainly results from the nitro group. Under our experimental conditions the mutagenicity of PNHA was higher at the same concentrations than that of NHA, although the percentage of nitrogen in PNHA was lower than that in NHA. Humic materials are the product of the decay of organic matter and their constituent and structure vary with their origin. Therefore, this result may be due to the different constituents of their original humic materials.

Pesticides or herbicides containing nitro groups such as fenitrothion (4-nitro-m-tolyldimethylphosphorothioate), parathion (4-nitrophenyldiethylphosphorothioate), EPN (4-nitrophenylethylphenylphosphonothioate), dinoseb (2-secondary-butyl-4,6-dinitrophenol), DNOC (2,4-dinitro-o-cresol), trifluralin (α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) and nitrofen (2,4-dichlorophenyl-p-nitrophenylether) were used in our environment. It has been reported that the reduction of the nitro group of these compounds to the amino group by bacteria may lower their effectiveness as a pesticide or a herbicide and also lower their toxicity to humans and animals. Therefore, the nitro group of the nitrohumic acid may be reduced to the amino group in the soil and its mutagenicity may also be lowered. Studies on the metabolism of the nitrohumic acid in the soil are in preparation.

We have also examined whether the nitrohumic acid is mutagenic in S. typhimurium TA102 (a base-pair substitution mutation strain) and TA96 (a frameshift mutation strain) which contain adenine-thymine base pairs at the site of the mutation. Massaro et al. (1983) have reported that nitropyrenes as well as several other nitroarenes show considerable mutagenicity in TA102 and TA96. The treatment of lignites and brown coals with nitric acid produces not only nitrohumic acids but also exidized constituents of humic materials. TA102 detects a variety of oxidative mutagens including hydroperoxides and quinones, and a variety of aldehydes including formaldehyde, glyoxal, kethol and glutaraldehyde (Levin et al. 1982; 1984). NHA and PNHA were considered to be potent mutagens in TA102. However, NHA and PNHA showed a slightly mutagenic activity in TA102 and a little higher mutagenic activity in TA96. It may be considered as a reason that the mutagenicity test system without S9 mixtures was used. This result may also show that NHA and PNHA do not contain oxidative mutagens and mutagenic aldehydes which largely contribute to the mutagenicity in TA102. HA also did not show the significant mutagenicity in TA102 and TA96 (Table 2). The mutagenicity of the nitrohumic acid in TA96 was higher than that in TA102. It is suggested that under our experimental conditions the mutagenicity of NHA and PNHA results from the nitro group.

Nitrohumic acids were found to be mutagenic in all the strains used in this experiment and showed potent mutagenicity especially in TA98.

Acknowledgments. The authors are grateful to Professor Bruce N. Ames, University of California at Berkeley for the gift of the Salmonella typhimurium tester strains used in this study. The authors are also grateful to Miss K.Koizumi and Mr. M.Ishizaki for skillful assistance.

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Received May 9, 1987; Accepted June 17, 1987.