Synergistic Effect of Naphthoquinones on the Mutagenicity of Nitroarene

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Nitro reduction is a critical step in the mutagenic activation of nitroarene. Nitroarene and quinone are known to be reduced by common enzymes, and thus, naphthoquinone (NQ) was studied for its effects on the mutagenicity of nitroarene in the Ames test using *Salmonella typhimurium* TA98 without S9. The mutagenicity of 1,3-dinitropyrene in TA98 was found to increase 9- and 6-fold as much in the presence of 70 nmol/plate of 2-methyl-1,4-NQ and 2-hydroxy-1,4-NQ, respectively. Mutagenicity also became greater in 1,3,5-trinitronaphthalene, 1-nitropyrene and 3-nitrofluoranthene. Seventy nmol/plate of 2-methyl-1,4-NQ increased the mutagenicity of 1-nitropyrene by 10.5-fold as much.

Key words nitroarene; naphthoquinone; mutagenicity

Nitroarenes are environmental pollutants, most of which, particularly polycyclic nitroarenes, are carcinogenic and/or mutagenic. They have been detected in coal fly ash, carbon black photocopier toner, diesel exhaust and cigarette smoke, etc.^{1–8)} Naphthoquinones (NQs) are also widely distributed in nature as biological components or products of animals, plants and bacteria. For example, lapachol (2-hydroxy-(3-methyl-2-butenyl)-1,4-NQ) is derived from the heartwood of Asian and South American bignoniaceous plants. Lawsone (2-hydroxy-1,4-NQ) is from the leaves of *Lawsonia inermis* and it is still widely available as a natural hair dye in the U.S. Juglone (5-hydroxy-1,4-NQ) is from walnut shells and is found in fish and mammals. Phylloquinone (2-methyl-3-(3,7,11,15-tetramethyl-1,4-NQ) and menadione (2-Methyl-1,4-NQ) are known as coagulate vitamins. Menadione was chemically synthesized from β -methyl-naphthalene by the oxidation of chromic oxide, and the inhibitory action of the mutagenicities of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and aflatoxin B₁ were reported.^{9,10)} Dichlon (2,3-dichloro-1,4-NQ) was used as an agricultural chemical. 1,4-NQ and anthraquinone were detected in environmental pollution caused by diesel exhaust and cigarette smoke.^{11–13)} Nitroarenes and quinones are frequently found together and undergo reduction by reductases, such as DT-diaphorase, NADPH-cytochrome c reductase, NADH-cytochrome b₅ reductase and xanthine oxidase. The contribution of various chemicals to mutagenicity and carcinogenicity is a matter of concern,^{14,15)} and examination was thus made of the effects of NQs on the mutagenicity of nitroarenes in the Ames test using *Salmonella typhimurium* (*S. typhimurium*) TA98 tester strains.

MATERIALS AND METHODS

Chemicals 3-Nitrofluoranthene (3-NFR), 2-nitrofluorene (2-NF), 1,3-dinitropyrene (1,3-DNP), 1,2-NQ, 5-hydroxy-1,4-NQ, 2-hydroxy-1,4-NQ, 4-amino-1,2-NQ hemihydrate, 1,2-NQ-4-sulfonic acid potassium salt, 2-anilino-1,4-NQ, 2-hydroxy-(3-methyl-2-butenyl)-1,4-NQ, 5,8-dihydroxy-1,4-NQ, 2,3-dichloro-1,4-NQ, and 2,3-dichloro-5,8-dihydroxy-1,4-NQ were purchased from Aldrich Chemical Company, Inc. 1,4-NQ and anthraquinone (AQ) were from Kanto Chemical Co., Inc. 1,3,5-Trinitronaphthalene (1,3,5-TNN) was synthesized with the nitration of naphthalene and the

structure was confirmed by mass spectrometry and 400 MHz proton NMR by Dr. K. Fukuhara, NIHS (Japan). 2-Methyl-1,4-NQ, 2-methyl-3-(3,7,11,15-tetramethyl)-1,4-NQ, and all other chemicals were obtained from Wako Pure Chemical Industries, Ltd., Japan.

Mutagenicity Assay in the *S. typhimurium* Strain *S. typhimurium* strain TA98 was kindly provided by Dr. T. Nohmi, NIHS, Tokyo (Japan). A *Salmonella* mutagenicity assay was carried out according to the method of Ames *et al.*¹⁶⁾ with pre-incubation.¹⁷⁾ Quinones were dissolved in acetone and dimethylsulfoxide (DMSO) followed by the addition of 50 μ l quinone solution. A mixture of harvested strains and sodium phosphate buffer and then 50 μ l nitroarene solution in DMSO were added to this system, followed by pre-incubation at 37°C for 20 min. Each number of revertant colonies per plate is counted in the mean of two experiments in duplicate.

RESULTS AND DISCUSSION

The structures of 14 NQs are shown in Fig. 1 and the results of the effects of these 14 NQs on the mutagenicity of 1,3-DNP are listed in Table 1. The mutagenicity of the NQs alone could hardly be detected in TA98,¹⁸⁾ while that of the nitroarenes was clearly noted to increase in the presence of NQs. 2-Methyl-1,4-NQ had a marked effect on the mutagenicity of 1,3-DNP. The mutagenic activity of 1,3-DNP without NQ was 91.5 revertants/plate, while, with 7 and 21 nmol/plate of 2-methyl-1,4-NQ, the activity increased to 186 and 693 revertants/plate respectively. The mutagenicity increased up to 828 revertants/plate with 70 nmol/plate of 2-methyl-1,4-NQ by 9 times as much. 2-Hydroxy-1,4-NQ, 4-NH₂-1,2-NQ and 2-anilino-1,4-NQ increased the mutagenicity of 1,3-DNP by 5.8-, 3.2- and 2.0-fold, respectively. Increases by the other NQs were less than 2-fold in all cases. NQs, which enhance the mutagenicity of 1,3-DNP, possess electro-donating groups such as methyl, hydroxyl, amino and anilino groups on the quinone-ring.

The effect of 2-methyl-1,4-NQ and 2-hydroxy-1,4-NQ on the mutagenicity of other nitroarenes such as 1,3,5-TNN, 2-NF, 3-NFR and 1-NP were examined and the results are shown in Table 2. 2-Methyl-1,4-NQ and 2-hydroxy-1,4-NQ enhanced the mutagenicity of 3-NFR, 1-NP and 1,3-DNP.

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Fig. 1. Structure of the 14 NQs

Table 1. Mutagenicity of 1,3-DNP with NQs

Naphthoquinones	Revertant colonies		
	7 nmol/plate rev./plate	21 nmol/plate rev./plate	70 nmol/plate rev./plate
1,2-NQ	109 (1.2)	115 (1.3)	82 (0.9)
1,4-NQ	111 (1.2)	76 (0.8)	14 (0.2)
5-Hydroxy-1,4-NQ	97 (1.1)	83 (0.9)	0 (0.0)
2-Hydroxy-1,4-NQ	179 (2.0)	275 (3.0)	523 (5.7)
4-Amino-1,2-NQ hemihydrate	135 (1.5)	177 (1.9)	291 (3.2)
1,2-NQ-4-sulfonic acid potassium salt	104 (1.1)	96 (1.0)	106 (1.2)
2-Anilino-1,4-NQ	122 (1.3)	140 (1.5)	184 (2.0)
2-Methyl-1,4-NQ	186 (2.0)	693 (7.6)	828 (9.0)
2-Hydroxy-3-(3-methyl-2-butenyl)-1,4-NQ	120 (1.3)	128 (1.4)	139 (1.5)
5,8-Dihydroxy-1,4-NQ	80 (0.9)	88 (1.0)	92 (1.0)
2,3-Dichloro-1,4-NQ	75 (0.8)	27 (0.3)	0 (0.0)
2,3-Dichloro-5,8-dihydroxy-1,4-NQ	73 (0.8)	42 (0.5)	0 (0.0)
2-Methyl-3-(3,7,11,15-tetramethyl)-1,4-NQ	103 (1.1)	86 (0.9)	80 (0.9)
AQ	110 (1.2)	108 (1.2)	101 (1.1)

The number of revertants per plate with 1 ng/plate of 1,3-DNP was 91.5. Increase was expressed as the ratio of the number of revertants to 1 ng/plate of 1,3-DNP in a parenthesis. Mean background revertant strain TA98 was 13. The number of revertants per plate with 14 NQs were less than 16.

For example, the mutagenicity of 1-NP at 4 ng/plate without NQ was 47.5 revertants/plate. The revertant colonies of 1-NP with 21 nmol/plate of 2-methyl-1,4-NQ was 308 revertants/plate and that with 70 nmol/plate of 2-methyl-1,4-NQ was 497 revertants/plate. Thus, 2-methyl-1,4-NQ at 70 nmol/plate increased the mutagenicity of 1-NP by 10.5 times as much. However, the increase in the mutagenicity of 2-NF by

2-hydroxy-1,4-NQ and 2-methyl-1,4-NQ was less than 2-fold. In the case of 1,3,5-TNN, the increase in mutagenicity by 2-hydroxy-1,4-NQ was also less than 2-fold. The mutagenicity of stronger mutagens such as 1,3-DNP, 1-NP was significantly enhanced by NQs. Nitroarenes possessing strong mutagenicity are easily reduced¹⁹⁾ and their mutagenicity readily increases. The results of Table 1 suggest the

Table 2. Mutagenicities of Nitroarenes with NQs

Nitroarenes	rev./nmol	2-Hydroxy-1,4-NQ		2-Methyl-1,4-NQ	
		21 nmol/plate rev./plate	70 nmol/plate rev./plate	21 nmol/plate rev./plate	70 nmol/plate rev./plate
1,3,5-Trinitronaphthalene	5	52 (1.3)	44 (1.1)	114 (3.0)	83 (2.1)
2-Nitrofluorene	76	116 (1.1)	114 (1.1)	128 (1.2)	143 (1.3)
3-Nitrofluoranthene	2349	299 (2.7)	392 (3.1)	338 (2.6)	514 (4.0)
1-Nitropyrene	5656	182 (3.8)	237 (5.0)	308 (6.5)	497 (10.5)
1,3-Dinitropyrene	26741	275 (3.0)	523 (5.8)	693 (7.6)	828 (9.0)

Revertants per plate with 2 µg/plate 1,3,5-trinitronaphthalene was 38.5; 300 ng/plate 2-nitrofluorene was 107.5; 5 ng/plate 3-nitrofluoranthene was 128.0; 4 ng/plate 1-nitropyrene was 47.5; 1 mg/plate 1,3-dinitropyrene was 91.5. An increase in ratios compared with nitroarene alone are shown in parentheses.

NQs, which easily give electrons,¹¹⁾ enhanced the mutagenicity of 1,3-DNP. Hakura *et al.* reported that NQs were mutagenic on *S. typhimurium* TA104, 2673, 100, 97 and 102, and these are due to oxidative damage produced with active oxygen species, which are generated as a result of the reduction of the NQs, and to bulky NQ-DNA adducts, accounting for their electrophilic property, whose contribution was largely dependent on the substituents of NQs.¹⁸⁾ Studies are now underway to clarify this mechanism by examining quinone reduction and the superoxide-generation of NQs with TA98.

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