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Tadashi Okabayashi, Misao Ide, Akihiro Yoshimoto, and Miyuki Otsubo: Mutagenic Activity of 4-Nitroquinoline 1-Oxide and 4-Hydroxyaminoquinoline 1-Oxide on Bacteria.

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Although numerous reports have dealt with the mutagenic activity of 4-nitroquinoline 1-oxide (4NQO), the usefulness of this compound in the study of bacterial genetics is limited because of its weak mutagenicity on bacteria.[1] The only exception is the work of Mashima and Ikeda who showed that 4NQO was one of the most powerful mutagens on a strain of a *Streptomyces* species.[2] This microorganism, however, has multicellular property, which makes it inconvenient for the genetic study. In this communication we wish to demonstrate that 4NQO is also a potent mutagen on unicellular bacteria. It is also shown that 4-hydroxyaminoquinoline 1-oxide (4HAQO), a microbial reduction product of 4NQO and is mutagenic on *Aspergillus niger*,[3] is also mutagenic on *Escherichia coli*.

Throughout this study a reverse mutation test was performed to assay the mutagenic activity. *E. coli* WN-22 (Pro^-) was obtained from *E. coli* W (ATCC 9637) by a penicillin method[4] after 4NQO treatment and used as a marker strain. The mutant cells were grown in a nutrient broth for 4 hour at 37° with shaking. The cells (about 7×10^9 cells/ml.) were harvested by centrifugation and washed once with a phosphate buffer (0.06*M*, pH 7.2). The washed cells were resuspended either in the phosphate buffer (in the case of 4NQO treatment and ultraviolet irradiation) or in 0.06*M* KH_2PO_4 in 0.9% sodium chloride (in the case of 4HAQO treatment), so as to give the final cell densities of 1×10^9 cells/ml. or 5×10^9 cells/ml. Treatment with 4NQO and 4HAQO was performed by allowing the cells to stand in test tubes at 28° for the appropriate period. As a control, ultraviolet irradiation was carried out with a 15 W germicidal lamp at a distance of 30 cm. Survival ratio was determined on bouillon agar plates. The Pro^+ revertants were scored on the Davis[5] medium agar plate after 3 days incubation at 37°.

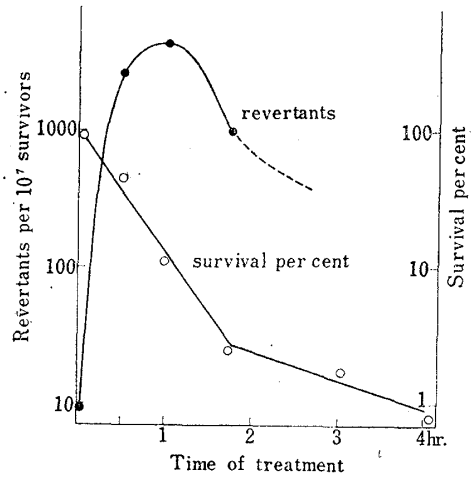

Fig. 1. Survival and the Number of Revertants of *E. coli* WN-22 (Pro^-) after Treatment with 4NQO

The number of spontaneous mutants were 0.2/10^7 cells.

Figure 1 shows one example in which 1×10^9 cells/ml. of *E. coli* WN-22 was treated with 35 μg./ml. of 4NQO. Survival number decreased as a function of time. Initially a sharp increase is observed in the ratio of the number of revertants to the number of survivors, but then this ratio decreases as the viable cell number decreases.

Table I shows another typical example. In this case 4NQO and 4HAQO treatments were

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Table I. Mutagenic Effect of 4NQO and 4HAQO on *E. coli*

Treatment	5×10^9 cells/ml. Survival per cent	5×10^9 cells/ml. Pro$^+$ revertants per 10^7 survivors	Treatment	1×10^9 cells/ml. Survival per cent	1×10^9 cells/ml. Pro$^+$ revertants per 10^7 survivors
4NQO[a] (μg./ml.)			4NQO[b] (μg./ml.)		
0	100	0.2	0	100	0.2
25	83.7	455	5	86.2	85.6
50	33.2	3420	10	40.9	1190
75	1.8	8860	15	13.2	3760
100	1.1	4080	20	4.4	6470
4HAQO·HCl[c] (μg./ml.)					
0	100	3.7			
25	73.5	21.0			
50	19.9	104			
75	6.5	945			
100	0.9	3420			
UV (sec.)			UV (sec.)		
0	100	0.2	0	100	0.18
10	94.5	195	10	44.7	700
15	59.6	540	15	15.1	2050
20	49.0	740	20	3.4	2380
25	38.7	1040	25	0.4	480
30	32.8	1300			

a) 4NQO (20 mg.) were dissolved in 10 ml. of acetone and aliquots of suitable dilutions in the phosphate buffer were added to the cell suspensions.
b) 4NQO was dissolved in the phosphate buffer and added to cell suspensions.
c) 4HAQO·HCl (2 mg.) were dissolved in 1 ml. of 75% ethanol and added to the cell suspensions. Ethanol concentration of all reaction mixtures including control run was carefully equalized. Although the standing of cells in the absence of 4HAQO in this condition caused considerable autodecay, no correction was made for this phenomenon and the survival number obtained without added 4HAQO was taken as 100%.

performed at 28° for 20 hour. The table shows that both 4NQO and 4HAQO are at least as powerful as ultraviolet light in the present reverse mutation system.

We have many unpublished experiences which failed to induce reverse mutation of bacteria with 4NQO. The unsuccessful results may at least partly be due to the use of unsuitable marker strains. The choice of *E. coli* WN-22, a 4NQO induced mutant, is based on the fact that some mutants induced by a mutagen can easily be reverted by the same mutagen.[6] The present work shows that 4HAQO is also mutagenic on *E. coli* WN-22. This may support the view that microbial reduction of 4NQO to 4HAQO participates in the manifestation of the mutagenicity of 4NQO.[3]

4NQO can also induce the mutation of *Brevibacterium liquefaciens*. Preliminary experiments in reverse mutation test with the 4NQO-induced auxotrophs of *B. liquefaciens* gave rather complicated results. However, at least some mutants are also reverted by 4NQO at a high frequency.

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