SHORT COMMUNICATION

REPAIR OF DNA DAMAGED BY 4-NITROQUINOLINE 1-OXIDE: A COMPARISON OF *ESCHERICHIA COLI* AND BACTERIOPHAGE T4 REPAIR SYSTEMS

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4-Nitroquinoline 1-oxide (4NQO), which is one of the most widely used chemical carcinogens, induces damages in DNA *in vivo* (Endo *et al.* 1970). It has been shown that *uvrA* and *uvrB* mutants of *Escherichia coli*, which are unable to repair ultraviolet (UV)-induced pyrimidine dimers, are lethal to 4NQO (Kondo *et al.* 1970). The function of *uvrA* and *uvrB* genes of *E. coli* is similar to that of *v* gene of bacteriophage T4; these genes control the formation of endonucleases which are responsible for the first incision step of excision repair of UV-irradiated DNA (Yasuda and Sekiguchi 1970; Taketo *et al.* 1972; Braun and Grossman 1974).

In this paper we report that regardless of their similarity in the function in repair of UV-damaged DNA the *v* and the *uvr* gene products act differently on 4NQO-induced damages. The *v* mutant is as resistant to 4NQO as is the wild type T4 whereas the *uvr* mutants exhibit increased sensitivity to the drug. It was shown, moreover, that T4 endonuclease V, which is coded by the *v* gene and introduces strand breaks in UV-irradiated DNA, does not act on 4NQO-treated DNA.

Sensitivity of uvr mutants of E. coli to 4NQO

Cross sensitivity between UV and 4NQO has been shown with *uvrA* and *uvrB* mutants of *E. coli* strain B (Kondo *et al.* 1970). We have examined 4NQO sensitivity of various *uvr* mutants, derived from K12 strains. A part of the results is shown in Fig. 1, which confirms the finding of Kondo *et al.* (1970) and further indicates that *uvrC* mutants are also sensitive to 4NQO. In spite of their high sensitivity to UV, *uvrD* mutants exhibit no increased sensitivity to 4NQO.

From these results it is suggested that in *E. coli* there are common steps for the repair of damages induced by UV and by 4NQO and that such steps are controlled by *uvrA*, *uvrB* and *uvrC* genes. The *uvrD* gene products, which is involved in the repair of UV-induced damage, is not concerned with the repair of 4NQO-induced damage.

Effect of 4NQO on infectivity of T4

4NQO does not inactivate free phage; no decrease in infectivity was observed when T4D or v_1 was incubated with 100 $\mu\text{g/ml}$ of 4NQO in broth at 37°C for 60 minutes.

Fig. 1. Sensitivity of *uvr* mutants to 4NQO. Bacteria were grown at 37°C in broth and plated on nutrient agar (20 ml) containing various amounts of 4NQO. The plate was incubated at 37°C overnight. ×—×, W3623 (wild type); □—□, N18-5 (*uvrA*); ■—■, N3-1 (*uvrB*); ●—●, N4-3 (*uvrC*); ○—○, N16-1 (*uvrC*); ⊙—⊙, N17-7 (*uvrC*); △—△, N13-1 (*uvrD*); ▲—▲, N10-1 (*uvrD*). The mutants were derivatives of W3623 (Ogawa *et al.* 1968). Similar results were obtained with AB1886 (*uvrA*) and AB2499 (*uvrB*), derived from AB1157.

This is probably due to the fact that an enzymic activation is necessary for the action of 4NQO to DNA (Tada and Tada 1975).

When bacteria infected with T4D or v_1 were exposed to 4NQO during the intracellular growth of phage, the formation of infective phage was equally inhibited (Fig. 2A). On the other hand, v_1 exhibited higher sensitivity to UV when either free phage or phage-infected cells were irradiated (Fig. 2B). It should be noted that *E. coli* AB2499 (*uvrB*) was used as host and indicator to exclude the possibility of an involvement of the host endonuclease in the process. These results suggest that the *v* gene is not concerned with the repair of 4NQO-induced damages in T4 DNA.

Action of T4 endonuclease V on 4NQO-treated DNA

Friedberg (1972) reported that T4 endonuclease V does not degrade DNA which has been treated with 4NQO *in vitro*. To obtain more definite evidence, we have performed experiments using DNA treated with 4NQO *in vivo*. *E. coli* AB2499 (*uvrB*) was incubated with 100 µg/ml of 4NQO for 60 minutes under the growth conditions and DNA was extracted. As a control DNA was prepared from untreated cells and irradiated with UV.

Figure 3 shows sedimentation patterns in alkaline sucrose gradient of 4NQO-treated

Fig. 2. Sensitivity of T4 and v_1 to UV and 4NQO. (A) 4NQO sensitivity: *E. coli* AB2499 (*uvrB*) was grown in broth to a concentration of 2×10^8 cells/ml and infected with T4D or v_1 at a multiplicity of 0.1. Free phages were inactivated by anti-T4 serum. Various concentrations of 4NQO was added to infected cells 5 minutes after infection. After incubation at 37°C for 12 minutes, the culture was diluted by 10^{-3} in broth and incubated at 37°C for additional 40 minutes. Few drops of chloroform was added to the cultures, and lysates were diluted and plated on *E. coli* AB2499. ●—●, T4D; ○—○, T4 v_1 . (B) UV sensitivity: T4D- or v_1 -infected *E. coli* AB2499 was irradiated at 9 minutes of infection. The dose rate of UV was approximately 2 J/m²/sec. Cultures were diluted into broth and incubated at 37°C for additional 40 minutes. After addition of chloroform, lysates were diluted and plated on AB2499. ●—●, T4D; ○—○, T4 v_1 . Dotted lines represent survival curves of free phage, irradiated prior to infection. ▲---▲, T4D; △---△, T4 v_1 .

and UV-irradiated DNA after incubation with an extract of T4D-infected and v_1 -infected cells. The v gene-specific fragmentation of UV-irradiated DNA is clearly seen, in agreement with the previous result (Yasuda and Sekiguchi 1970b). However, the same treatment of 4NQO-treated DNA does not result in the fragmentation of DNA. A similar result was obtained when 4NQO-treated DNA was incubated with a partially purified preparation (Fraction III) of T4 endonuclease V and then analyzed by neutral sucrose gradient after heat denaturation (data not shown).

From these results it is likely that T4 endonuclease V, which is capable of inducing strand interruptions in UV-irradiated DNA, does not act on 4NQO-damaged DNA. It is of interest to see whether *E. coli* endonuclease controlled by *uvrA* and *uvrB* genes induces strand breaks in 4NQO-treated DNA.

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Fig. 3. Sedimentation in alkaline sucrose of 4NQO-treated and UV-irradiated DNA after treatment with an extract of T4D-infected or *v*₁-infected cells. (A) 4NQO-treated DNA: *E. coli* AB2499 (*uvrB*) was grown at 37°C in M9 medium containing [¹⁴C]thymine and then exposed on 100 μg/ml of 4NQO for 60 minutes under the growth conditions. DNA was extracted by phenol treatment. (B) UV-irradiated DNA: [¹⁴C]thymine-labeled DNA, prepared from *E. coli* AB2499 was irradiated at 200 J/m². The two types of DNA's were incubated at 37°C for 30 minutes with an extract of T4D-infected or *v*₁-infected *E. coli* 1100 (15 minutes after infection). The reaction mixture (0.5 ml) contains 2.5 μg of [¹⁴C]-thymine-labeled DNA (25,000 c.p.m.), 20 μmoles of Tris-HCl (pH 7.5), 25 μmoles of EDTA (pH 7.5) and 25 μg protein of extract. The reaction was terminated by shaking with phenol. DNA (0.1 ml) was overlayed on 4.7 ml of 5-20% sucrose gradient prepared in 0.02 M K₃PO₄-0.05 M EDTA (final pH, 12.6) and centrifuged for 3 hours at 36,000 r.p.m. at 15°C. ●—●, Treated with T4D-infected cell extract; ○—○, treated with T4*v*₁-infected cell extract; ×—×, untreated.

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