Genetic Characterization of Adenine-3 Mutants Induced by 4-Nitroquinoline 1-Oxide and 4-Hydroxyaminoquinoline 1-Oxide in *Neurospora crassa*¹

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**SUMMARY**

Specific locus mutations induced by the chemical carcinogens, 4-nitroquinoline 1-oxide (4NQO) and 4-hydroxyaminoquinoline 1-oxide (4HAQO), have been characterized to obtain a presumptive identification of the genetic alterations at the molecular level. One hundred eighty-four 4NQO-induced and 219 4HAQO-induced ad-3 mutants of *Neurospora crassa* obtained in previous studies were studied with a series of genetic tests that permits determination of their genotype and the frequencies of point mutations and multilocus deletions. These tests have shown that the spectrum of ad-3 mutations among 4NQO-induced mutants is similar to that of 4HAQO-induced mutants. None of the 4NQO- or 4HAQO-induced mutants is a multilocus deletion mutant. The ratio of ad-3A to ad-3B mutants is the same in the two samples, as well as the frequencies of complementing ad-3B mutants. These data suggest, then, that the mechanism of mutation induction by 4NQO in *N. crassa* is identical to that of 4HAQO. It is not clear, however, whether 4NQO is mutagenic per se or reduction of 4NQO to 4HAQO is the first step involved in the mutagenesis of this compound in *Neurospora*.

The heterokaryon tests have shown that the relatively high frequencies of 4NQO- or 4HAQO-induced ad-3B mutants show allelic complementation and that most of the complementing ad-3B mutants (74% of 4NQO induced and 71% of 4HAQO induced) have nonpolarized complementation patterns. From this we conclude that both agents induce predominantly base-pair substitution mutations in *N. crassa*. The results are in agreement with our other studies which show that potent chemical carcinogens induce predominantly base-pair substitution mutations in *N. crassa*.

**INTRODUCTION**

The potent chemical carcinogen 4NQO³ is known to interact with DNA, transform mammalian cells, cause chromosomal aberrations in mammalian cells, and induce gene mutations in many organisms (see Ref. 11 for review). 4NQO can be reduced enzymatically to 4HAQO in microorganisms (29). Enzymes involved in reduction of 4NQO to 4HAQO have also been found in various animal tissues (36). The reduced form, 4HAQO, also possesses a potent carcinogenic activity (9, 35). Due to similarities in the biochemical and biological properties of 4NQO and 4HAQO (10, 21, 26, 28), the latter compound has been suggested as the active form of 4NQO carcinogenesis.

In previous studies with the ad-3 (adenine-3) test system, we showed that there is a positive correlation between mutagenicity in *N. crassa* and carcinogenicity in laboratory mammals among the chemical carcinogens studied (32) and that potent chemical carcinogens induce predominantly base-pair substitution mutations (24, 31, 33). Both 4NQO and 4HAQO are potent mutagens in *N. crassa* (25). It is of interest to know whether 4NQO and 4HAQO cause similar spectra of genetic alterations in *N. crassa*. In the ad-3 system of *N. crassa*, one can isolate mutants and characterize them by a series of genetic tests as described by de Serres (4, 5) (Chart 1). Using these tests, point mutations and multilocus deletions covering one or both ad-3 loci can be distinguished (4, 6, 7). The point mutations can be further characterized by determining the frequencies of allelic complementation among the ad-3B mutants (2, 5, 8). On the basis of this information, a presumptive identification of the genetic alterations at the molecular level can be made. With this assay system, the spectra of genetic alterations caused by 4NQO and 4HAQO or other chemical carcinogens can be compared, and in this report we present data that show that both chemicals produce identical spectra of genetic alterations and that both compounds induce predominantly base-pair substitution mutations in *N. crassa*.

**MATERIALS AND METHODS**

**Strains.** One hundred eighty-four of the 190 4NQO-induced and 219 of the 222 4HAQO-induced ad-3 mutants of *N. crassa* from forward-mutation experiments reported earlier (25) were made homokaryotic for their adenine requirement. These mutants were then characterized genetically by complementation, dikaryon, and trikaryon tests.

**Complementation Tests.** The complementation pattern and the genotype (ad-3A, ad-3B or ad-3A ad-3B) of each

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³ The abbreviations used are: 4NQO, 4-nitroquinoline 1-oxide; 4HAQO, 4-hydroxyaminoquinoline 1-oxide.

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muatant were determined by heterokaryon tests for complementation. The methods of Brockman and de Serres (2) were followed except that only the following 9 testers were used: Tester 1, ad-3B, complon 1 (2-17-258); Tester 2, ad-3B, complon 2 (2-17-123); Tester 3, ad-3B, complons 10-11 (2-31-8); Tester 4, ad-3B, complon 15 (2-32-3); Tester 5, ad-3B, complons 16-17 (2-32-5); Tester 6, ad-3A, (1-68-13); Tester 7, noncomplementing ad-3B (1-112-2); Tester 8, hist-2 (histidine-2), nic-2 (nicotinic acid-2), al-2 (albinos-2) (74-OR33-3A); Tester 9, ad-2 (adenine-2), inos (inositol) (74-OR60-44A). Testers 1 through 5 determined the complementation patterns of ad-3B mutants showing allelic complementation (Chart 2). On the basis of the complementation patterns, ad-3B mutants were classified as noncomplementing, nonpolarized complementing, and polarized complementing. Testers 6 through 8 determined the genotype. Tester 9 was used as a positive control to test the ability of each mutant to form a heterokaryon.

Dikaryon Test. Dikaryon tests were carried out by platting conidia from each mutant in Fries’ minimum medium (14) supplemented with adenine and pantothene. The plates were incubated at 35° for 2 days and were then examined for the presence or absence of cot (colonial temperature-sensitive) colonies. The ad-3 mutation was induced in Component II of the heterokaryons, which carries the genetic markers cot, al-2, and pan-2 (pantothene acid-2). The presence of cot colonies indicates that the induced ad-3 mutants are viable in the medium supplemented with adenine. This type of mutant is classified as a point mutation (ad-3R) (3), a mutant which is reparable on medium supplemented with adenine. Mutants that gave no viable cot colonies in the minimum medium supplemented with adenine were subjected to the trikaryon tests.

Trikaryon Test. The ad-3 mutants that gave a negative dikaryon test either carried a point mutation within the ad-3A or ad-3B locus and an independent recessive lethal mutation at another locus elsewhere in the genome (ad-3R + RL) or carried a multilocus deletion covering 1 or both ad-3 loci and 1 or more adjacent essential genes (ad-3Rm). These 2 types of mutants can be distinguished by trikaryon test. The methods have been reported elsewhere by de Serres (4, 6).

RESULTS

Complementation Test. The results of the complementation tests are summarized in Tables 1 and 2. These data show that 33% of the 4NQO-induced mutants are ad-3A and that 67% are ad-3B mutants. Among 124 4NQO-induced ad-3B mutants, 63% show allelic complementation; among the 78 complementing ad-3B mutants, 74% have nonpolarized complementation patterns.

Among 219 4HAQO-induced ad-3 mutants, 37% are ad-3A and 63% are ad-3B mutants. A total of 68% of the 4HAQO-induced ad-3B mutants show allelic complementation, and that 67% are ad-3B mutants. Among 124 4NQO-induced ad-3B mutants, 63% show allelic complementation; among the 78 complementing ad-3B mutants, 74% have nonpolarized complementation patterns.

Chart 1. The ad-3 mutation was induced by 4NQO and 4HAQO in the component II of the genetically marked heterokaryon. The complementation and trikaryon testers were used for genetic analysis of the mutants induced by 4NQO and 4HAQO. □, extent of functional inactivation in each mutant.
Aspergillus niger, Okabayashi (28) has shown that mutants tissue culture cells are phenotypically identical to those carcinogens produce exclusively point mutations in the ad-3 either the ad-3A or ad-3Bm testers, none of the mutants similar patterns of repair kinetics (16). It has been reported and 4HAQO in vivo have shown that both compounds have ad-3A ad-3Bm tester: these are classified as ad-3'@ + RLCI induced by 4NQO (10). In studies on mutation induction in DISCUSSION

Studies on the repair of DNA damage induced by 4NQO and 4HAQO in vivo have shown that both compounds have similar patterns of repair kinetics (16). It has been reported that intranuclear inclusions induced by 4HAQO-HCl in tissue culture cells are phenotypically identical to those induced by 4NQO (10). In studies on mutation induction in Aspergillus niger, Okabayashi (28) has shown that mutants induced by 4NQO and 4HAQO are phenotypically similar. Both compounds are potent mutagens in N. crassa (25). The genetic characterization of ad-3 mutants induced by 4NQO and 4HAQO reported here shows that the relative proportion of ad-3A and ad-3B mutants induced by 4NQO is similar to that of 4HAQO-induced mutants. The frequencies of 4NQO-induced ad-3B mutants showing allelic complementation and the frequency of 4NQO-induced complementing ad-3B mutants showing nonpolarized complementation patterns are similar to the frequencies among 4HAQO-induced ad-3B mutants. None of the differences between 4NQO- and 4HAQO-induced mutants are statistically significant as determined by χ² test. Neither 4NQO nor 4HAQO induced ad-3A ad-3B double mutants. Furthermore, none of the 4NQO- or 4HAQO-induced ad-3 mutants are multilocus deletions covering either the ad-3A or the ad-3B locus and adjacent essential genes. These data indicate that 4NQO and 4HAQO only induce point mutations with identical spectra of genetic alterations at the molecular level. It seems, therefore, that the mechanism of mutation induction by 4NQO and 4HAQO is similar in N. crassa. However, it is not known whether 4NQO is mutagenic per se or whether the mutagenic activity of this compound is enhanced by, or requires, an enzymatic reduction process. In vitro studies (17, 37, 39) which show that 4HAQO, not 4NQO, inactivates bacteriophage and induces single-stranded breaks in DNA, seem to suggest that the conversion of 4NQO to 4HAQO is necessary for certain biological activity of 4NQO in some prokaryotes. 4NQO induces predominantly GC to AT base-pair transition mutations in T4 bacteriophage (18, 19). This compound has been shown to revert base-pair substitution mutants in yeast (34). In N. crassa, 63% of 4NQO-induced and 68% of 4HAQO-induced ad-3 mutants show allelic complementation; 74% of 4NQO-induced and 71% of 4HAQO-induced complementing ad-3B mutants have nonpolarized complementation patterns. Data of Mailing and de Serres (22, 23) have shown that there is a correlation between complementation pattern and genetic alteration. Ad-3 mutants that are induced by chemicals that cause predominantly base-pair substitution mutations have high frequencies of ad-3B mutants showing allelic complementation and nonpolarized complementation patterns. By inference, then, the data from 4NQO- and 4HAQO-induced mutants suggest that both compounds induce predominantly base-pair substitutions in N. crassa. Induction of base-air substitutions might be expected since the interaction of 4NQO and 4HAQO with DNA probably is a
covalent binding of these compounds with purine bases (15, 27, 30), rather than an intercalation of the compounds between bases (20, 38). Binding of 4NQO or 4HAQO to purine residues in DNA could cause base-pair substitution mutations following mispairing during DNA replication. However, if a binding of 4NQO or 4HAQO to DNA causes depurination of DNA, other types of mutations might also be found. It is also interesting to note that the mechanism of mutation induction by 4NQO in Salmonella typhimurium appears to be different from that in phage, Saccharomyces cerevisiae or N. crassa, since this compound reverts frameshift mutation mutants of S. typhimurium with much higher efficiency than base-pair substitution mutants (1, 12, 13). The data presented here clearly indicate that in the eukaryotic organism N. crassa, both potent chemical carcinogens, 4NQO and 4HAQO, induce exclusively point mutations and the same relative frequencies of genetic alterations at the molecular level.

Ad-3 mutants induced by both agents have high frequencies of ad-3B mutants showing allelic complementation and nonpolarized complementation patterns. This result is in agreement with our studies which have shown that ad-3B mutants induced by potent chemical carcinogens have high frequencies of nonpolarized complementation patterns (24, 31, 33) (Table 4). Thus, our studies with N. crassa not only indicate that there is a positive correlation between mutagenicity and carcinogenicity but also indicate that potent chemical carcinogens produce mutations predominantly by base-pair substitution.

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REFERENCES

Induction of ad-3 mutants by 4NQO and 4HAQO in N. crassa


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