Mutagenicity of a Monofunctional Alkylating Agent Derivative of Acridine in Neurospora

Abstract. *Purple adenine* (ad-3) *mutants induced in* Neurospora crassa *by 2-methoxy-6-chloro-9-(3-[ethyl-2-chloroethyl] aminopropylamino) acridine dihydrochloride (ICR-170) result from intragenic alterations. They are further characterized by low frequencies of leakiness, allelic complementation, and nonpolarized complementation patterns. These characteristics distinguish them from* ad-3 *mutants induced by x-rays, nitrous acid, and 2-aminopurine.*

Two-armed or difunctional alkylating agents are generally more effective inhibitors of tumor growth than their one-armed or monofunctional analogs (*1*). A monofunctional nitrogen mustard derivative of acridine, ICR-170 (Fig. 1), has been shown, however, to possess potent activity against ascites tumors in mice at molar dosages comparable to those needed for the corresponding difunctional form (*2*). Furthermore, ICR-170 (*3*) is highly mutagenic without causing apparent chromosome breakage at the dumpy locus of *Drosophila* (*4*). It is an interesting mutagen because its two components—an acridine nucleus and a monofunctional nitrogen mustard—are mutagens as separate molecules (*5*).

Recent studies by de Serres and coworkers show that certain characteristics (such as the frequency of leakiness, allelic complementation, and types of complementation patterns) of the purple adenine (*ad-3*) mutants of *Neurospora crassa* vary as a function of mutagenic origin (*6*). It has been suggested that these characteristics differ because the single-gene mutants induced by a given mutagen are predominantly either base-pair substitutions or base-pair additions and deletions (*6*). A special heterokaryon permits an additional characteristic, the incidence of inactivation of two or three linked loci, to be studied; these alterations are probably deletions and therefore indicate the occurrence of gross chromosomal changes as opposed to point mutational (intragenic) events (*7–9*). We show in this report that ICR-170 is a potent mutagen in *Neurospora* and that the characteristics of *ad-3* mutants induced by this agent are different from those induced by x-rays (*6, 9*), nitrous acid (*6, 10*), or 2-aminopurine (*6, 11*) and from those of spontaneous (*6, 11*) origin.

The *ad-3* mutants were induced in component II of heterokaryotic (dikaryotic) conidia in which component I is *A, hist-2, ad-3A, ad-3B, nic-2*, + ; *ad-2*; + ; *inos*; +; and component II is *A*, +,+,+,+ , *al-2*; + ; *cot*; + ; *pan-2* (*7, 12*). Conidia, 1×10^6 per milliliter in distilled water, were treated with 0, 1, or 5 μg of ICR-170 per milliliter for 0, 2, 4, 6, and 8 hours. All procedures with ICR-170 and conidia were performed under red light to eliminate the photodynamic action of the acridine nucleus (*13*). The *ad-3* mutants induced in heterokaryotic conidia were recovered by a direct method (*14*) modified as previously described (*11*) and by the addition of 10 μg of niacinamide per milliliter and 0.1 percent Difco purified agar. With this technique, mutation at the *ad-3* region of component II of a heterokaryotic conidium gives a purple colony while nonmutant colonies are white. An adenine-requiring dikaryon was isolated from each purple colony by selective plating and was maintained on minimal medium containing adenine and niacinamide. These dikaryons containing *ad-3* mutants induced in component II were used in all tests described here. Since both *ad-3* mutants in component I are nonleaky and noncomplementing (*15*), the presence of this component has no influence on the characterization of the *ad-3* mutations in component II.

No *ad-3* mutants were recovered from the control population of 3.9×10^6 colonies; more extensive tests conducted previously gave a spontaneous frequency of *ad-3* mutants of 0.38×10^{-6} (*11*). Among conidia treated with 1 μg and 5 μg of ICR-170 per milliliter for 8 hours, the *ad-3* mutation frequencies were 187 and 2287 per 10^6 survivors, respectively. At these two concentrations, 84 and 28 percent survived, respectively.

The mutants (187) from the conidia treated with 1 μg of ICR-170 per milliliter were selected for analysis because they carried fewer morphological variations. The growth response of each mutant was assayed in duplicate alone and with the following tester strains: (i) *ad-3A*; (ii) *ad-3B*, noncomplementing; (iii) *nic-2*; (iv) *ad-3B*, complon 1 (*16*); (v) *ad-3B*, complon 2; (vi) *ad-3B*, complon 16-17; (vii) *ad-3B*, complon 17; and (viii) *ad-2, inos*. The tests were conducted in test tubes (13 by 100 mm) containing 2

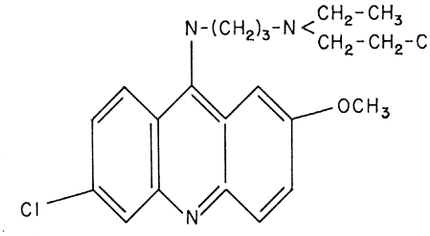

Fig. 1. ICR-170; 2-methoxy-6-chloro-9-(3-[ethyl - 2 - chloroethyl] aminopropylamino) acridine dihydrochloride.

ml of Fries' basal medium plus 1.5 percent sucrose at 30°C for 12 days. The final conidial concentration was 1×10^5/ml. Tester (viii) is a control that gave a positive heterokaryon growth response with all *ad-3* mutants. Testers (iv) through (vii) are from a more complete set of testers that define 17 complementing units (*17*) or complons (*16*) of the *ad-3B* locus.

According to the response of the mutants with testers (i) to (iii), each was classified as either *ad-3A; ad-3B; ad-3A, ad-3B; ad-3A, ad-3B, nic-2*; or *ad-3B, nic-2*. These three loci are closely linked with about 0.1 to 0.2 crossover units in the *ad-3A–ad-3B* interval and 3.0 units between *ad-3B* and *nic-2* (*18*). The last three classes result from the inactivation of two or three of these linked loci. They will be referred to as deletion types, since the data from homology tests (*8*) and analysis of their induction kinetics (*9*) suggest that they result from chromosomal aberrations such as deletions. Deletion types were not found among nitrous acid–induced *ad-3* mutants (*10*), but about one-third of the *ad-3* mutants induced by x-rays were of these types (*7, 15*). All of the ICR-170–induced *ad-3* mutants were either *ad-3A* (57 out of 187) or *ad-3B* (130 out of 187); therefore, ICR-170, like nitrous acid, in-

Table 1. Characteristics of *ad-3* mutants of various mutagenic origins.

Mutagenic origin	Frequency (%)		
	Leaky *ad-3* mutants among total	Complementing *ad-3B*'s among *ad-3B*'s	Nonpolarized *ad-3B*'s among complementing *ad-3B*'s
ICR-170	5.3	23.8	35.5
X-ray*	5.0	25.0	39.1
Nitrous acid*	27.8	72.1	83.8
2-Aminopurine*	39.1	77.1	92.6
Spontaneous*	9.4	38.1	25.0

* Data from reference 6 (*ad-3* mutants induced in wild type 74A).

duces only intragenic changes at this region of chromosome I of *Neurospora*.

The tubes inoculated with the ICR-170–induced mutants alone give the frequency of leakiness among all *ad-3* mutants, and the growth responses with testers (iv) to (vii) give the incidence of allelic complementation and of complementation pattern types among the *ad-3B* strains. Only 5.3 percent (10 out of 187) of the mutants were leaky. Thirty-one out of 130 *ad-3B* mutants complemented at least one of the *ad-3B* testers [testers (iv) to (vii)], giving a frequency of 23.8 percent complementing and 76.2 percent non-complementing *ad-3B* mutants. The complementing *ad-3B* mutants can be further classified as polarized or non-polarized (*6*) on the basis of their response with testers (iv) to (vii). Mutants that do not complement testers (i) and (ii), but do complement testers (iii) and (iv) or only (iv) give a polarized pattern—that is, they map from complon 1 to some distance to the right. The nonpolarized mutants are usually less extensive and can show a variety of patterns (*11*). Among the complementing *ad-3B*'s induced by ICR-170, 64.5 percent (20 out of 31) were polarized and 35.5 percent (11 out of 31) were nonpolarized.

In Table 1 the characteristics of the ICR-170–induced *ad-3* mutants are contrasted with previously described characteristics of *ad-3* mutants isolated in wild-type 74A from four other origins (*6*). It is interesting that the ICR-170–induced mutants are very similar to those induced by x-rays and unlike those induced by 2-aminopurine or nitrous acid in the three characteristics shown. On the other hand, if the incidence of deletion types is considered, the ICR-170–induced mutants are similar to those induced by nitrous acid (*10*) and unlike those induced by x-rays (*7, 9, 15*). Purple adenine mutants induced by ICR-170 are, therefore, unique among the *ad-3* mutants of different mutagenic origins analyzed to date. Furthermore, the low incidences of leakiness, allelic complementation, and of nonpolarized complementation patterns suggest that the enzyme(s) specified by the *ad-3* region is absent or grossly altered in ICR-170–induced *ad-3* mutants. Two explanations of these low incidences can be offered: (i) the mutations are predominantly base-pair additions or deletions rather than base-pair substitutions, and ICR-170 is therefore acting like an acridine (*19*); or (ii), the mutations are predominantly base-pair substitutions giving primarily nonsense codons (*20*) or alternatively mis-sense codons (*20*) at a specific set of sites within the *ad-3* region that specify especially crucial amino acids for determining an active enzyme.

The high mutagenicity of this compound is striking. It would be interesting to know if both components of the molecule are mutagenic in *Neurospora* or if there is a potentiation of the mutagenic activity of the alkylating agent by the acridine nucleus by virtue of its strong affinity for DNA (*21*). Comparative mutation studies of the two components at the *ad-3* region of *Neurospora* should answer this question.

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12. Locus abbreviations: *A*, sex (mating type); *hist*, histidine; *ad*, adenine; *nic*, nicotinic; *inos*, inositol; *al*, albino; *cot*, colonial temperature-sensitive; *pan*, pantothenic acid.
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Immunoassay for Human Chorionic "Growth Hormone-Prolactin" in Serum and Urine

Abstract. *Chorionic "growth hormone-prolactin" in serum and urine from pregnant women, in serum from umbilical cord, and in amniotic fluid was assayed by a sensitive immunological method dependent on the combination of rabbit antiserum to the growth hormone-prolactin with the iodine-131–labeled hormone. The hormone is detectable in serum and urine early in gestation, and with advancing pregnancy its concentration in serum continues to increase to a maximum during the last trimester. It was not found in serum within 8 hours after delivery.*

Human placenta contains a protein which cross-reacts with rabbit antiserum to human pituitary growth hormone (HGH) (*1–6*), and a similar protein has been reported in simian placenta (*3–5*). The placental protein fraction giving an incomplete cross-reaction with antiserum to HGH and exhibiting prolactin-like and luteotropic activity has been called "placental lactogen" by Josimovich and MacLaren (*1*). It was suggested that the placental protein has activity similar to that of growth hormone, the potency of the placental hormone being appreciably less than that of pituitary growth hormone (*3, 4*). We have proposed that this hormone which appears to function as an important metabolic hormone of pregnancy be tentatively designated chorionic "growth hormone-prolactin" (CGP) (*3, 4*). Studies in vivo and in vitro, including production by cultures of human chorionic villi, provide firm evidence that the placenta synthesizes and secretes CGP (*4*). Although exhibiting