

Genotoxic activity of 2-amino-N-hydroxylaminopurine (AHA) in *Aspergillus nidulans*

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In *Aspergillus nidulans*, as well as in other eukaryotic cells, not all base analogs are mutagenic. For example, 2-aminopurine (2-AP) is non-mutagenic or weakly mutagenic for eukaryotes while it is mutagenic for bacteria.

Because of their potential use in genetical research, an effort has been made to find base analogs mutagenic for eukaryotic cells. Work in this field has been successful: in fact, 6-hydroxylaminopurine (HAP) and 2-amino-N-hydroxylaminopurine (AHA) have been found mutagenic for yeast as well as for other eukaryotic cells. (Pavlov et al. 1991, Mut. Res. 253:33-46). In particular, Brockman et al. (Mut. Res. 177:61-75, 1987) tested the mutagenic activity of HAP and AHA in *Neurospora crassa* and found that AHA is about equally mutagenic as HAP at low doses but more mutagenic at high doses.

In this paper we report the genotoxic activity of AHA in *A. nidulans*. In this mold, we have tested AHA-induced lethality and mutagenic and recombinogenic effects.

We only summarize briefly the technical approach followed in our experiments; for more detailed information see Babudri et al. 1993, Mut. Res. 321:19-26.

1. Germinating conidia (6.5 h of incubation) were treated with AHA for 1.5 h in soft agar medium (0.45% agar in liquid minimal medium).
2. The mutagenic activity was tested by selecting p-fluorophenylalanine resistant (FPAR) mutants on minimal medium plus FPA.
3. Only colonies that grew and conidiated well were regarded as true FPAR mutants. FPA resistance may be due to mutations arising at several loci: therefore, to estimate the frequency of mutation at one locus (*fpaA*) we tested resistance of FPAR colonies on minimal medium (pH 4.5) plus 3-amino-L-tyrosine and phenyl-anthranilic acid. On this medium, only *fpaA* mutants are viable (Calvori and Morpurgo, 1966, Mut. Res. 230:187-195).
4. Recombinational activity as tested by selecting *fpaA/fpaA* colonies (resistant to FPA) arising from *+fpaA* diploids (sensitive to FPA).

Mutagenic activity of AHA was tested in 4 experiments with AHA doses ranging from 1 µg/ml to 10 µg/ml.

In Table 1 we report the mutagenic potency of AHA evaluated as *fpaA* mutants per viable cell and the percentage of survival with respect to the controls. A clear dose-effect relationship was not evident in the range of doses tested: for example, note in the Table the frequency obtained at 10 µg/ml and the similar values obtained at 1 and 2.5 µg/ml.

As for recombinogenic activity of AHA, no enhancement was observed in AHA-treated conidia, compared with controls (Table 2).

On the basis of these results, we can conclude that AHA is mutagenic (but not lethal) for *A. nidulans* germinating conidia.

When we compare the data with AHA to those obtained with HAP in *Aspergillus* (Babudri et al. 1993, Mut. Res. 321:19-26) this main conclusion can be drawn: AHA is a less potent mutagen than HAP in *A. nidulans* in the dose range tested, a result which is opposite to that obtained in *Neurospora* (Brockman et al. 1987, Mut. Res. 177:61-75) but is in agreement with that obtained by Pavlov et al. (Mut. Res. 1991, 253:33-46) with the yeast *Saccharomyces cerevisiae*. It must be noted that we have compared the mutagenic potency of HAP and AHA at doses where the mutation frequency has already reached a plateau, notwithstanding the surviving fraction was not affected at all; in fact, statistical analysis of data obtained with AHA shows that the peak in mutation frequency at 5 ug/ml is not statistically significant.

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Table 1. Surviving fraction and frequency of AHA-induced *fpaA* mutants in a haploid strain of *Aspergillus nidulans*. The mean values of four experiments and the standard errors of the means are reported.

AHA dose (ug/ml)	fpaA mutants per viable cell	survivors (% of controls)
0	0.16 x 10 ⁽⁻⁵⁾ (+/- 0.18)	
1	4.80 x 10 ⁽⁻⁵⁾ (+/- 0.75)	95
2.5	5.40 x 10 ⁽⁻⁵⁾ (+/- 1.21)	102
5	15.50 x 10 ⁽⁻⁵⁾ (+/- 5.1)	93
10	3.00 x 10 ⁽⁻⁵⁾ (+/- 0.54)	89

Table 2. Frequency of AHA-induced FPA resistant recombinants selected at the *fpaA* locus in a diploid strain *fpaA*/+ (mean values from two experiments)

AHA dose (ug/ml)	fpaA/fpaA recombinants per viable cell
0	1.2 x 10 ⁽⁻⁴⁾
1	1.5 x 10 ⁽⁻⁴⁾
2	1.3 x 10 ⁽⁻⁴⁾