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CONVERSION OF A POWERFUL FRAMESHIFTER ACRIDINE TO A BASE-PAIR SUBSTITUTION ANALOG

Brenda R. Brown, William J. Firth and Lerena W. Yielding

Laboratory of Molecular Biology
University of Alabama in Birmingham
University Station
Birmingham, Alabama 35294

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SUMMARY

The frameshift mutagenic mechanism for acridines has been attributed to the intercalative type of association between acridines and nucleic acids. However, it appears that these molecular details are insufficient to explain the frameshifting process. In order to design an effective drug probe to analyze the in vivo interactions of acridines leading to frameshifting, an azide analog of 9-aminoacridine was studied in Ames' Salmonella strains. The surprising findings were that by substituting an amino group at the 9 ring position with an azido group, the mutagenicity was converted from frameshifter to base-pair substitution.

INTRODUCTION

The nature of the acridine interaction with nucleic acids has fascinated and eluded scientists for the past twenty years. The "frameshifting" mutagenicity of proflavine led to the elucidation of the nature of the triplet code. Yet in spite of the great amount of acridine research, the molecular details explaining 'frameshift mutagenesis' are still unknown.

9-Aminoacridine has been described as a classic intercalator and causes frameshift mutations in E. coli (1), Salmonella typhimurium (2) and bacteriophage T4 r II (3). Over the years, the interaction of 9-aminoacridine with nucleic acids has been examined by the vast array of in vitro techniques available to science, the culmination coming with the x-ray crystallographic descriptions of 9-aminoacridine complexed with dinucleotides (4,5). However, the details of the in vivo molecular interaction are as yet obscure. The reason for this inability to determine the exact interaction stems from the non-covalent

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nature of most drug-nucleic acid associations. The reversibility of the complex formed between the drug molecule and its target makes the isolation and characterization of the in situ interaction virtually impossible.

The adaptation of the technique of photoaffinity labeling for the study of drug-nucleic acid interactions has recently been undertaken in this laboratory (6-14). If the reversible drug-DNA complex can be converted to an identical but irreversible one, it can then be isolated and studied. This is accomplished by synthesis of azide analogs of the drug molecule; when photolytically activated, a covalent linkage is formed between the drug and the site of interaction.

In order to aid in the elucidation of the molecular mechanism responsible for the frameshifts caused by 9-aminoacridine, its analog, 9-azidoacridine, was synthesized. In light of results obtained from similar work with ethidium bromide (13), this analog was expected to covalently attach in the same orientation as the parent compound, and thus provide a probe to study the acridine in vivo interaction.

MATERIALS AND METHODS

9-Aminoacridine was purchased from Sigma and recrystallized from acetone. 9-Azidoacridine was synthesized by the procedure of Reynolds (15), and was characterized by its infrared and mass spectral properties. All solutions were prepared in dimethyl sulfoxide (Certified Spectranalyzed, Fisher Scientific) just prior to their use in experiments.

Mutagenicity Assays. S. typhimurium strains developed by Bruce N. Ames, University of California at Berkeley, were kindly provided us by him, and were used to test the mutagenic activity of the compounds. The strains are histidine auxotrophs, and a summary of the genetic traits of the strains used is presented in Table I. Bacterial manipulation was carried out essentially as recommended by Ames (2,16,17). Overnight cultures of nutrient broth (8 grams nutrient broth (Difco) + 5 grams NaCl/liter deionized H_2O) were grown to a density of 5 x 10^8 cells/ml. All strains were routinely checked for rfa character by testing for crystal violet sensitivity, and for continued histidine auxotrophy by checking for growth in the absence of histidine. 0.1 ml aliquots of a log phase bacterial culture were added to 2.5 ml top agar (0.5% agar + 0.6% NaCl) to which either 5 x 10^{-5}M histidine and biotin were added when looking for histidine revertants, or 1.5 x 10^{-3}M histidine and 5 x 10^{-5}M biotin, when checking for drug toxicity. The top agar suspension was then poured on bottom agar plates (Vogel-Bonner minimal salts + 2% glucose + 1.5% agar), and the drug (10λ of 2 mg/ml dimethyl sulfoxide) was placed in a well in the center of the poured plate. The plates were allowed to sit for 3 hours at room temperature in the dark to allow drug diffusion on the plate. Photolysis, where appropriate, was for 10 minutes using a General Electric 30W fluorescent lamp at 2 x 10^4 ergs/cm^2/sec. Each drug was plated in triplicate and each drug

TABLE I

GENETIC CHARACTERISTICS OF THE SALMONELLA TYPHIMURIUM STRAINS

Strain	Type of Reversion	Excision Repair
TA1537	Frameshift (-1 or +2)	-
TA1977	" "	+
TA1538	Frameshift (-2 or +1)	-
TA1978	" "	+
TA1535	Base pair substitution	-
TA1975	" " "	+

All strains are histidine auxotrophs which are reverted by a specific type of mutation. They all possess the rfa (deep rough) mutation, which enhances cell permeability to drugs.

was tested at least three different times. Controls of bacteria with dimethyl sulfoxide added, in the light and dark, were always carried out, and reversion counts corrected for background.

RESULTS

A summary of the results is presented in Table II. The effectiveness of 9-aminoacridine as a frameshift mutagen is seen by the high reversion rates in strain TA1537. It is similarly mutagenic for TA1977 indicating that the 9-aminoacridine-induced lesion is not repaired. It should be noted that 9-amino, as well as other acridines, are ineffective at reverting the other frameshift reversion strains TA1538/TA1978, or the base-pair substitution strains TA1535/TA1975.

The 9-azidoacridine analog did not prove to be at all effective as a frameshift mutagen as seen by its inability to revert strains TA1537/TA1977. It was, however, a powerful base-pair substitution mutagen as indicated by the high reversion rates in TA1535 (an excision repair deficient strain). Moreover, the excision repair strain TA1535 was not reverted by this azide analog. It should also be pointed out that 9-azidoacridine did not require photolytic activation in order to cause the reversion of TA1535, being equally effective

TABLE II

EFFECTS OF 9-AMINOACRIDINE AND 9-AZIDOACRIDINE OF SALMONELLA STRAINS

	TA1537		TA1977		TA1538		TA1978		TA1535		TA1975	
	His^+ revertants	Killing zone	His^+ revertants	Killing zone	His^+ revertants	Killing zone	His^+ revertants	Killing zone	His^+ revertants	Killing zone	His^+ revertants	Killing zone
9-Aminoacridine	500	9	500	7	0	7	0	6	0	7	0	0
9-Azidoacridine												
dk	0	9	0	14	0	6	0	7	1000	8	10	16
lt	10	10	10	11	0	9	0	7	1000	7	20	16
pre-photolyzed 9-Azidoacridine (acridine dimer)												
dk	0	0	0	0	0	0	0	0	0	0	0	0
lt	0	0	0	0	0	0	0	0	0	0	0	0

0.1 ml log phase culture was added to 2.5 ml top agar with appropriate additions of histidine and biotin. After the top agar was spread on glucose bottom agar plates, wells were made in the agar, to which 10 λ of a drug solution of 2 mg/1 ml DMSO was added. Plates were allowed to sit at room temperature in the dark for 3 hours and then irradiated where appropriate for 10 min. Control plates having only DMSO were always done, and this background count subtracted from the reversion counts. Toxicity was measured using identical technique, having only the higher histidine concentration. The zone of killing is in millimeters. The DMSO control had no zone of killing.

with or without photolytic activation. These findings appear to be highly significant. Since the 9-azido analog was not mutagenic for strain TA1975, it can be concluded that this acridine lesion was indeed repaired. Furthermore, this ability to be repaired is usually indicative of a covalent attachment, since non-covalent interactions are not repaired. Clearly, these results demonstrate that the 9-azido lesion is quite different from that of the 9-amino. Not only do both of these agents induce mutations of an exceedingly different nature (frameshift vs base-pair substitution), but one lesion (9-azido) is repaired while the other (9-amino) is not.

9-Azidoacridine is a highly reactive molecule which could explain the fact that photolysis is not required to activate its mutagenicity. Recent mass spectroscopy studies show that when a solution of 9-azidoacridine (2 mg/10 ml MeOH) is irradiated, the predominant species formed is the acridine dimer. Consequently, a sample of the photolysis product, the acridine dimer, was tested for its ability to revert the Salmonella strains, and no mutagenic activity or toxicity was observed (Table II). Since these Salmonella strains are all rfa mutants, and their cell walls are permeable by virtue of modified polysaccaride side chains, the lack of mutagenesis by the photolysis product is, therefore, probably not due simply to the lack of cell penetration.

DISCUSSION

Our results rule out the 9-azidoacridine as a useful drug molecule with which to study frameshift mechanism by the use of covalent attachment of the drug molecule to the DNA. Synthesis of additional azidoacridines is proceeding in our laboratory to find a more suitable drug analog with which to pursue the goal of understanding the molecular mechanism responsible for frameshift mutagenesis.

The azido analog, however, should still be useful as a probe for studying drug mutagenesis, since a classic frameshifter acridine has been converted to a base-pair substitution mutagen behaving much like an alkylating agent. In previous work with another well known intercalator, ethidium bromide

(13), conversion of the amino group(s) to azide moieties had produced ethidium analogs which bound covalently and produced only frameshift mutations with no base-pair substitution activity. X-ray crystallographic studies comparing the crystal structures of ethidium monoazide and the ethidium cation complexed with 5-iodocytidylyl (3'-5') guanosine (18) and with 5-iodouridylyl (3'-5') adenosine (19) show the azide group easily accommodated within the dinucleotide structures (20), making analogous interaction of the ethidium monoazide and its parent ethidium possible. Analysis of the acridine drug-nucleic acid complex would allow a comparison of this interaction and the x-ray crystallographic structure proposed for the 9-aminoacridine-CpG complex (5). The x-ray data suggest a possible explanation for the altered mutagenicity, since the 9-aminoacridine appears to be intercalated between base-pairs in the DNA with its 9-amino group situated in a groove (three complexes have the 9-amino in the major groove, and one complex shows the 9-amino in the minor groove). If the acridine azide were interacting analogously to the parent acridine, the azide substituent would be located in the groove, and at the time of activation would not have a target on the DNA to attack. A more reasonable complex may be an acridine stacking on the outside of the DNA helix (similar to one already suggested for acridines (5)) where the azide group may have a number of target attachment sites.

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