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Mutagenicity of Acridines in a Reversion Assay Based on Tetracycline Resistance in Plasmid pBR322 in Escherichia Coli

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Fundamental and Molecular Mechanisms of Mutagenesis

Mutagenicity of acridines in a reversion assay based on tetracycline resistance in plasmid pBR322 in *Escherichia coli*

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Abstract

The mutagenicity of a series of acridine compounds was studied in an assay based on the reversion of mutations in the tetracycline-resistance gene (*tet*) of plasmid pBR322 in *Escherichia coli*. Mutations that restore the tetracycline-resistant phenotype were detected in tetracycline-sensitive strains carrying mutant plasmids. Mutations that revert by +2, +1, −1, and −2 frameshift mutations and by base-pair substitutions were used to analyze the mutagenicity of two simple acridines, two acridine mustards, and a nitroacridine. The simple acridines (9-aminoacridine and quinacrine) effectively induced −1 frameshifts and weakly induced +1 frameshifts. The acridine mustards (quinacrine mustard and ICR-191) were more potent inducers of −1 and +1 frameshifts than the simple acridines. Reactive acridines, including both the mustards and the nitroacridine Entozon, were effective inducers of −2 frameshifts but the simple acridines were not. The two classes of reactive acridines differed from one another, in that the mustards were better inducers of +1 frameshifts than Entozon, whereas Entozon was a particularly potent inducer of −2 frameshifts. None of the compounds induced +2 frameshifts, and the induction of base-pair substitutions was negligible. These results confirm and extend studies showing that adduct-forming acridines are stronger frameshift mutagens than simple intercalating acridines and that the acridines differ from one another not only in overall mutagenic potency but also in the prevalence of different classes of frameshift mutations.

Keywords: Acridine; *E. coli*; Frameshift; pBR322; Plasmid mutagenesis; Tetracycline resistance

1. Introduction

Genetic effects of acridine compounds have been studied in microorganisms, insects, plants, cultured mammalian cells, and mammals (Ferguson and Denny, 1991). Most notable among their mutagenic effects is the induction of frameshift mutations, including both base-pair deletions and additions. Simple acridines such as 9-aminoacridine (9AA) associate with DNA noncovalently by intercalation, whereas reactive acridines, including the acridine mustards and nitroacridines, both intercalate and form covalent adducts in DNA (Ferguson and Denny, 1991; McCoy et al., 1981). Reactive acridines tend

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to be more potent mutagens than the simple intercalators (Brown et al., 1980; Ferguson and Denny, 1991; McCoy et al., 1981).

We measured the mutagenicity of five acridines in a reversion assay based on mutations in the tetracycline-resistance gene (*tet*) of pBR322 in *E. coli*. The mutations, which reside in a 276-base-pair *Bam*HI-*Sal*I restriction fragment, were isolated in a forward mutation assay (Fuchs et al., 1988) or were constructed specifically for the detection of a particular mutational event (Burnouf and Fuchs, 1985). In the forward mutation assay, bacteria are transformed with pBR322 containing chemically-induced premutational lesions in the target restriction fragment, and mutants are identified as tetracycline-sensitive (Tc^s) transformants (Fuchs et al., 1988). Forward mutation spectra of several mutagens have been analyzed by isolating and sequencing the fragment (Bichara and Fuchs, 1985; Burnouf et al., 1987; Hebert et al., 1988; Hoffmann and Fuchs, 1990; Koffel-Schwartz et al., 1984). In the reversion assay, mutant plasmids are treated in vitro (Koffel-Schwartz and Fuchs, 1989; Maenhaut-Michel et al., 1992) or in a bacterial host (Bintz and Fuchs, 1990; René et al., 1988), and revertants are detected by positive selection for the tetracycline-resistant phenotype (Tc^r). In the present study, we used the latter treatment procedure, exposing the plasmids to the mutagens in vivo.

The reversion assay uses mutants whose reversion mechanisms have been characterized in earlier studies (Burnouf and Fuchs, 1985; René et al., 1988) and are summarized in Table 1. Analysis of the size of restriction fragments has shown that plasmid pXVIII, which contains a +1 frameshift, reverts by a −1 frameshift, and that plasmid pXIX, which contains a −1 frameshift, reverts by a +1 frameshift (René et al., 1988). Sequence analysis of 15 revertants of plasmid pW17, which is identical to pXVIII in having an additional guanine in the run of G:C base pairs at site 536–540, revealed that 14 were −1 frameshifts that restore the nonmutant sequence and that the single exception had a −1 frameshift at a site immediately 5′ to the run of guanine residues (Maenhaut-Michel et al., 1992). In the case of pX_1, the insertion of a CG dinucleotide in the *NarI* sequence (GGCGCC) at site 414 of the *tet* gene creates a *Bss*HI restriction site. When strains containing pX_1 revert to the Tc^r phenotype, the *Bss*HI site disappears and the *NarI* site is restored, indicating that the CG dinucleotide was deleted (Burnouf and Fuchs, 1985).

The *tet* reversion assay has been used to study the induction of −2 frameshifts in alternating GC sequences by *N*-acetoxy-*N*-2-acetylaminofluorene (*N*-Aco-AAF) and other agents that similarly form covalent adducts at the C8 position of guanine (Bintz and Fuchs, 1990). It has also been used to explore the roles of genes of the SOS regulon in the induction by

Table 1
Strains of *Escherichia coli* and plasmids

Strain [a]	Plasmid	Alteration in mutant	Position of alteration	Sequence context in mutant	Reversion mechanism [b]	Plasmid genotype [c]
BR871	pX_1	+CG	414	5′ GGCGCGCC 3′	−2	(1,2)
BR872	pb1	−CG	414	5′ GGCC 3′	+2	(1,2)
BR878	pXVIII	+G	536–540	5′ GGGGGG 3′	−1	(1)
BR879	pXIX	−G	536–540	5′ GGGG 3′	+1	(1)
TS1800	pw120–29Pt	T → C	606	5′ CCTAC 3′	b.p.s.	(3)
AB1157pX_1	pX_1	+CG	414	5′ GGCGCGCC 3′	−2	(1,2)
AB1157pXVIII	pXVIII	+G	536–540	5′ GGGGGG 3′	−1	(1)
AB1157pXIX	pXIX	−G	536–540	5′ GGGG 3′	+1	(1)

[a] The strains with the BR prefix and TS1800 are plasmid-containing derivatives of ES548/7 (René et al., 1988), which is a phage T7-resistant mutant of ES548 exhibiting increased permeability to chemicals. The genotype of ES548 is *trpA540 rpsL lacZ(ICR36)* (René et al., 1988) and that of AB1157 is *thr-1 ara-14 leuB6 Δ(gpt-proA)62 lacY1 tsx-33 supE-44 galK2 λ⁻ rac⁻ hisG4(Oc) rfbD1 mgl-51 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1* (Bachmann, 1987).

[b] The frameshift mutations are classified by the number of base pairs gained or lost in the reversion process; b.p.s. = base-pair substitutions.

[c] All plasmids are Tc^S mutants of pBR322. They are described in the following papers: (1) René et al., 1988; (2) Burnouf and Fuchs, 1985; (3) Burnouf et al., 1987.

N-Aco-AAF of −1 frameshifts in runs of G:C base pairs and −2 frameshifts among alternating G:C base pairs (Koffel-Schwartz and Fuchs, 1989; Maenhaut-Michel et al., 1992). René et al. (1988) studied the mutagenicity of 9AA in the reversion assay using plasmids containing defined +1, −1, +2, and −2 frameshift mutations in strain ES548/7, which contains a mutation conferring resistance to phage T7 and greater permeability to chemicals than wild-type.

We compared the mutagenicity of two classes of reactive acridines with that of simple intercalators in the *tet* reversion assay using the same plasmids as René et al. (1988). We also included a plasmid that reverts by base-pair substitution. The compounds studied were the simple acridines 9AA and quinacrine, the acridine mustards ICR-191 and QM, and the nitroacridine Entozon. Structures are shown in Fig. 1. In the course of studying the mutagenicity of acridines, we explored the performance of the *tet* reversion assay, tested for the possibility of artifacts, and compared the responsiveness of T7-resistant bacteria with that of bacteria with wild-type permeability.

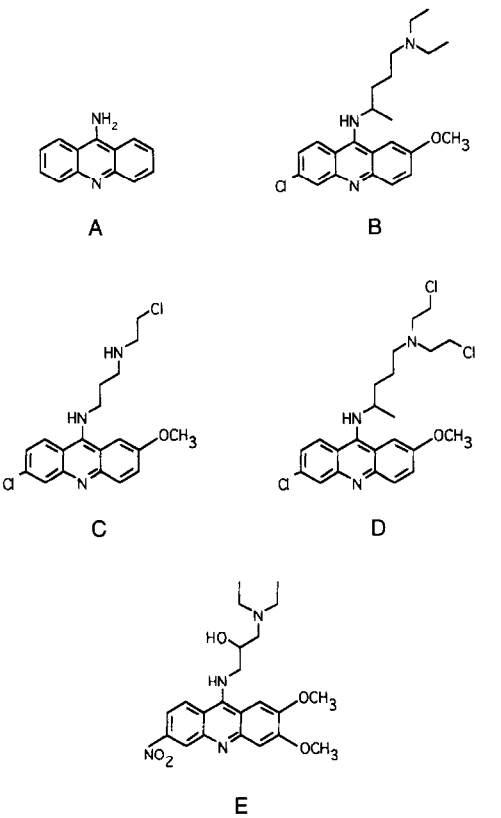

Fig. 1. Structures of acridine compounds: the simple intercalators 9-aminoacridine (A) and quinacrine (B); the acridine mustards ICR-191 (C) and quinacrine mustard (D); and the nitroacridine Entozon (E).

2. Materials and methods

2.1. Plasmids and bacterial strains

E. coli strains and pBR322 derivatives with mutations in the *Bam*HI-*Sal*I fragment of the *tet* gene are shown in Table 1. Strain TS1800 and the AB1157 derivatives were constructed by transformation. All other strains and plasmids are from the collection of R.P.P. Fuchs, CNRS, Illkirch, France.

2.2. Media and culturing

The growth medium was LB (Sambrook et al., 1989). LB was supplemented with ampicillin (50 μg/ml) to select for pBR322 and its Tc^s derivatives or with tetracycline (15 μg/ml) to select for Tc^r revertants. Cultures were initiated by streaking from soft agar or frozen stocks on LB + ampicillin. Single colonies were isolated and grown ~ 16 h at 37°C in liquid LB + ampicillin. Grown cultures were maintained at 4°C and used for several weeks. Genotypes of all cultures were confirmed by streaking on appropriate media, checking the spontaneous revertant frequency, and verifying the permeability of T7-resistant strains and a T7-sensitive control with crystal violet as described by Maron and Ames (1983). The same method was used with quinacrine to confirm the greater sensitivity of T7-resistant strains to acridines.

2.3. Transformation

Plasmid-bearing derivatives of AB1157 were constructed by $CaCl_2$ transformation (Sambrook et al., 1989). Briefly, lyophilized plasmid DNA was diluted to 1 ng/μl in TCM buffer and mixed 1:2 with competent cells (10^{10} cells/ml). Transformants were selected by spreading 3 μl of the cell-DNA mixture in 100 μl TCM on LB + ampicillin. Strain TS1800

was constructed by electrotransformation (Dower et al., 1988) of strain ES548/7 with plasmid pw120-29Pt. Immediately after pulsing with a Gene Pulser apparatus (Bio-Rad Laboratories. Richmond, CA), the cells were swirled in SOC medium (2% Bacto Tryptone, 0.5% Bacto Yeast Extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, and 20 mM glucose) for 30 min at 37°C and plated on LB + ampicillin to select for transformants.

2.4. Chemicals

Chemical structures are shown in Fig. 1. Quinacrine (quinacrine dihydrochloride; Chemical Abstracts Service Number [CAS] 69-05-6), QM (quinacrine mustard dihydrochloride; CAS 4213-45-0), 9AA (9-aminoacridine hydrochloride; CAS 134-50-9), and β-propiolactone (CAS 57-57-8) were purchased from Sigma Chemical Company (St. Louis, MO). ICR-191 dihydrochloride (CAS 17070-45-0), obtained from Dr. Herman Brockman, was from Terochem Laboratories (Edmonton, Alta., Canada). Entozon dihydrochloride (CAS 73972-50-6) was obtained from Dr. Herbert Rosenkranz, University of Pittsburgh; it had been synthesized by Hoechst AG, Frankfurt/Main, Germany.

2.5. Mutagenesis

A characterized culture was subcultured (10 μl into 5 ml) in LB containing the mutagen and grown in the dark in a shaker for 16 h at 37°C. To select for revertants, 0.1 ml of culture was spread on LB + tetracycline. Dilutions were spread on nonselective medium to quantify viable cells. Relative plating efficiencies (i.e., plate counts in the presence of a chemical divided by those in its absence) were calculated as an indication of toxicity. Cells were plated on LB ± ampicillin so that plasmid loss could be detected if it occurred. All plating was in triplicate, and colonies were counted after 30 h at 37°C. Results are reported as numbers of Tc^r colonies per 10^8 viable cells ± standard errors.

3. Results

Table 2 shows the revertibility of strains containing mutations in the *tet* gene by the simple intercala-

Table 2
9-Aminoacridine: induction of reversion to tetracycline resistance in tetracycline-sensitive mutants of pBR322 in *Escherichia coli*

Strain and reversion mechanism	Concentration (μg/ml)	Relative plating efficiency(%)	Tc^r revertants per plate	Revertant frequency ($Tc^r/10^8$ cells)
BR871	0	100	0.0	0.0 ± 0.0
(−2)	4	62	0.0	0.0 ± 0.0
	8	19	0.0	0.0 ± 0.0
	16	8	0.7	5.7 ± 2.9
BR872	0	100	0.0	0.0 ± 0.0
(+2)	4	111	0.0	0.0 ± 0.0
	8	29	0.0	0.0 ± 0.0
	16	1	0.0	0.0 ± 0.0
BR878	0	100	5.0	5.6 ± 0.0
(−1)	4	18	19.3	121.0 ± 25.5
	8	16	27.0	186.0 ± 14.0
	16	4	13.0	347.7 ± 30.9
BR879	0	100	0.3	0.3 ± 0.3
(+1)	4	72	3.7	4.4 ± 1.1
	8	44	7.0	13.6 ± 2.3
	16	13	1.3	8.9 ± 8.9
TS1800	0	100	1.0	1.0 ± 0.6
(b.p.s.)	4	34	0.0	0.0 ± 0.0
	8	4	0.0	0.0 ± 0.0
	16	2	0.0	0.0 ± 0.0
	32	0.9	0.0	0.0 ± 0.0

Table 3
Quinacrine: induction of reversion to tetracycline resistance in tetracycline-sensitive mutants of pBR322 in *Escherichia coli*

Strain and reversion mechanism	Concentration (μg/ml)	Relative plating efficiency(%)	Tc^r revertants per plate	Revertant frequency ($Tc^r/10^8$ cells)
BR871	0	100	0.0	0.0 ± 0.0
(−2)	16	61	0.3	0.4 ± 0.4
	32	40	0.7	1.2 ± 0.6
	64	19	3.3	12.5 ± 1.2
BR872	0	100	0.0	0.0 ± 0.0
(+2)	16	30	0.0	0.0 ± 0.0
	32	23	0.0	0.0 ± 0.0
	64	4	0.0	0.0 ± 0.0
BR878	0	100	5.7	9.1 ± 0.5
(−1)	16	43	98.3	368.7 ± 25.2
	32	18	74.0	643.7 ± 47.9
	64	0.7	5.0	1231.0 ± 752.0
BR879	0	100	0.0	0.0 ± 0.0
(+1)	16	56	6.7	5.9 ± 1.3
	32	29	8.0	13.9 ± 4.4
	64	0.1	0.0	0.0 ± 0.0
TS1800	0	100	0.7	0.4 ± 0.4
(b.p.s)	8	58	0.3	0.4 ± 0.4
	16	29	0.3	0.7 ± 0.7
	32	36	1.7	2.4 ± 0.6
	64	7	0.0	0.0 ± 0.0

Table 4
ICR-191: induction of reversion to tetracycline resistance in tetracycline-sensitive mutants of pBR322 in *Escherichia coli*

Strain and reversion mechanism	Concentration (μg/ml)	Relative plating efficiency (%)	Tc^r revertants per plate	Revertant frequency ($Tc^r/10^8$ cells)
BR871	0	100	0.0	0.0 ± 0.0
(−2)	2	76	36.3	29.6 ± 4.2
	4	36	105.0	179.3 ± 28.2
	8	3	41.0	917.3 ± 51.7
BR872	0	100	0.0	0.0 ± 0.0
(+2)	2	85	0.0	0.0 ± 0.0
	4	31	0.0	0.0 ± 0.0
	8	0.4	0.0	0.0 ± 0.0
BR878	0	100	24.3	19.4 ± 10.5
(−1)	2	90	133.3	119.0 ± 36.0
	4	56	805.3	1150.4 ± 307.0
	8	6	798.0	11515.2 ± 2512
	16	0.03	6.3	15833.0 ± 4640
BR879	0	100	0.0	0.0 ± 0.0
(+1)	2	47	2421.3	3843.3 ± 238
	4	26	2621.3	7404.8 ± 483
	8	1.5	1548.0	78979.6 ± 1870
	16	0.1	379.0	344545.5 ± 17344
TS1800	0	100	0.7	0.6 ± 0.6
(b.p.s)	2	107	0.0	0.0 ± 0.0
	4	47	0.7	1.2 ± 1.2
	8	21	1.7	6.7 ± 3.5
	16	1	0.0	0.0 ± 0.0

tor 9AA. Effective mutagenesis occurred only in strain BR878, which reverts by a −1 frameshift. A weak mutagenic response was observed in strain BR879, which reverts by a +1 frameshift. Table 3 shows that quinacrine, another simple acridine, produced a pattern of reversion similar to that of 9AA. It effectively induced −1 frameshifts and only weakly induced +1 frameshifts. Though weak, the +1 responses observed with 9AA and quinacrine in strain BR879 were reproducible and statistically significant ($p < 0.05$) in analyses of variance.

Table 4 shows reversion of the same strains by ICR-191. The data indicate a more potent induction of +1 and −1 frameshifts by this reactive acridine than by the simple acridines 9AA and quinacrine. The ratio of +1 to −1 mutagenesis also differed, being much higher for ICR-191 than for the simple intercalators. The responses to ICR-191 were dose-dependent, and the frequencies of −1 and +1 revertants at high doses were greater than 10^{-4} and 10^{-3} per cell, respectively. Unlike the simple acridines, ICR-191 caused a highly significant (analysis of variance; $p < 0.01$) dose-dependent induction of −2 frameshifts, as indicated by strain BR871. Like the simple acridines, it was inactive in strains BR872 and TS1800, whose reversion mechanisms are a +2 frameshift and base-pair substitutions, respectively.

QM was a less potent mutagen than ICR-191 but produced a similar pattern of reversion. As shown in Table 5, it was most effective in inducing +1 and −1 frameshifts but also induced −2 frameshifts. Like ICR-191, it did not cause a significant increase in base-pair substitutions (ICR-191: $p = 0.08$; QM: $p = 0.34$). A known base-pair-substitution mutagen, β-propiolactone (Brusick, 1977), served as a positive control in strain TS1800 and gave revertant frequencies of 912 ± 39 revertants per 10^8 cells at 3.2 mM (56% survival) and 139000 ± 1295 revertants per 10^8 cells at 6.4 mM (0.4% survival).

The data in Table 6 indicate that Entozon resem-

Table 5
Quinacrine mustard: induction of reversion to tetracycline resistance in tetracycline-sensitive mutants of pBR322 in *Escherichia coli*

Strain and reversion mechanism	Concentration (μg/ml)	Relative plating efficiency(%)	Tc^r revertants per plate	Revertant frequency ($Tc^r/10^8$ cells)
BR871	0	100	0.3	0.2 ± 0.2
(−2)	8	79	0.3	0.3 ± 0.3
	16	59	2.7	2.8 ± 0.4
	32	53	35.0	41.2 ± 2.9
	64	8	62.0	480.3 ± 54.3
BR872	0	100	0.0	0.0 ± 0.0
(+2)	8	77	0.0	0.0 ± 0.0
	16	52	0.0	0.0 ± 0.0
	32	34	0.0	0.0 ± 0.0
	64	11	0.0	0.0 ± 0.0
BR878	0	100	6.0	4.8 ± 1.7
(−1)	8	68	188.7	222.3 ± 43.9
	16	90	802.7	716.7 ± 84.2
	32	50	1064.0	1716.0 ± 175.0
	64	5	420.3	6568.0 ± 537.0
BR879	0	100	0.0	0.0 ± 0.0
(+1)	8	73	410.0	405.9 ± 3.5
	16	65	1196.0	1334.8 ± 78.8
	32	19	2221.3	8350.8 ± 396
	64	3	1970.7	47486.7 ± 3073
TS1800	0	100	5.0	5.6 ± 0.6
(b.p.s)	8	82	4.7	6.4 ± 4.4
	16	118	3.0	2.9 ± 0.6
	32	46	1.3	3.2 ± 0.8
	64	10	0.7	7.5 ± 3.8

Table 6
Entozon: induction of reversion to tetracycline resistance in tetracycline-sensitive mutants of pBR322 in *Escherichia coli*

Strain and reversion mechanism	Concentration (μg/ml)	Relative plating efficiency(%)	Tc^r revertants per plate	Revertant frequency ($Tc^r/10^8$ cells)
BR871	0	100	0.0	0.0 ± 0.0
(−2)	2	85	533.0	750.7 ± 61.7
	4	80	633.7	960.3 ± 14.4
	8	52	781.3	1796.0 ± 134.0
	16	0.1	1.3	1671.0 ± 1105.0
BR872	0	100	0.0	0.0 ± 0.0
(+2)	2	108	0.0	0.0 ± 0.0
	4	86	0.0	0.0 ± 0.0
	8	40	0.0	0.0 ± 0.0
	16	25	0.0	0.0 ± 0.0
BR878	0	100	47.3	32.2 ± 5.7
(−1)	2	46	414.7	609.8 ± 37.6
	4	42	391.0	633.7 ± 22.1
	8	17	392.3	1556.8 ± 117.3
	16	0.1	58.7	31041.0 ± 3237.7
BR879	0	100	0.3	0.2 ± 0.2
(+1)	2	47	0.7	0.7 ± 0.4
	4	57	7.0	6.4 ± 2.1
	8	18	16.0	46.4 ± 1.7
TS1800	0	100	0.7	0.6 ± 0.6
(b.p.s.)	2	66	0.5	0.6 ± 0.6
	4	76	2.3	2.6 ± 1.5
	8	28	4.3	12.9 ± 4.0

bles the acridine mustards in being a potent inducer of −1 frameshifts but is more like the simple acridines in causing a relatively small, though reproducible and statistically significant ($p < 0.01$), increase in +1 frameshifts. Entozon's most distinctive property is its potent induction of −2 frameshifts in strain BR871. It had minimal effect in strain TS1800 and no effect in strain BR872.

Table 7
Induction by ICR-191 of reversion to tetracycline resistance in tetracycline-sensitive mutants of pBR322 in *Escherichia coli* strain AB1157 *

Strain and reversion mechanism	Concentration (μg/ml)	Relative plating efficiency(%)	Tc^r revertants per plate	Revertant frequency ($Tc^r/10^8$ cells)
AB1157pX_1	0	100	0.0	0.0 ± 0.0
(−2)	4	81	0.0	0.0 ± 0.0
	8	30	0.3	1.4 ± 1.4
	16	7	0.7	9.5 ± 9.5
AB1157pXVIII	0	100	0.0	0.0 ± 0.0
(−1)	4	56	1.7	2.5 ± 1.8
	8	18	8.3	44.3 ± 28.5
	16	2	23.0	830.0 ± 182.0
AB1157pXIX	0	100	0.0	0.0 ± 0.0
(+1)	4	73	6.3	8.6 ± 3.2
	8	40	64.7	168.7 ± 58.3
	16	6	119.0	2140.0 ± 81.2

* Comparative data for the more permeable strain ES548/7 are in Table 4.

Table 7 shows the frameshift mutagenicity of ICR-191 in strain AB1157, which lacks the T7-resistance mutation of strain ES548/7. The data show lower mutation frequencies and less toxicity than in ES548/7, reflecting the lesser permeability of strain AB1157 to the mutagen. The induction of −2 frameshifts is not detected in plasmid pX_1 in strain AB1157, unlike the clear response of pX_1 in the permeable strain BR871 (Table 4). Like ICR-191, QM (data not shown) produced weaker mutagenic responses in strain AB1157 than in ES548/7. As in ES548/7, QM was a less potent mutagen than ICR-191.

Reconstruction experiments in which a small number of Tc^r bacteria were mixed into a population of Tc^s bacteria were performed to test for selection artifacts under the conditions of the mutation experiments. Tc^r bacteria typically have no selective advantage in the absence of tetracycline. However, an occasional clone was found to be somewhat resistant to quinacrine; such a clone would have a selective advantage when treated with acridines. Analysis of many clones for quinacrine-resistance (Q^r) revealed that there is no association between the Q^r and Tc^r phenotypes and that Q^r correlates with resistance to crystal violet. Q^r colonies arise among both Tc^r and Tc^s strains derived from ES548/7, and the decreased permeability of the Q^r derivatives appears to be ascribable to reversion of the T7 mutation. Accumulation of such nonpermeable derivatives is avoidable by growing cultures from single colonies and confirming genotypes. There is no indication of selection favoring Tc^r revertants under the conditions that precede plating on tetracycline in mutagenesis experiments.

Table 8
Relative revertibility of frameshift mutations in the pBR322 *tet* gene in *E. coli* by acridines, acridine mustards, and Entozon

Strain	Reversion mechanism	Compound	Revertant frequency at constant toxicity *
BR878	−1 Frameshift	9-Aminoacridine	200
		Quinacrine	590
		ICR-191	8450
		Quinacrine mustard	4700
		Entozon	1230
BR879	+1 Frameshift	9-Aminoacridine	11
		Quinacrine	15
		ICR-191	37900
		Quinacrine mustard	24300
		Entozon	31
BR871	−2 Frameshift	9-Aminoacridine	3
		Quinacrine	8
		ICR-191	580
		Quinacrine mustard	320
		Entozon	3030

* $Tc^r/10^8$ cells calculated for a toxicity corresponding to 20% relative plating efficiency.

4. Discussion

We studied the mutagenicity of simple intercalating acridines and two kinds of reactive acridines in a reversion assay based on *tet* mutations in pBR322 in *E. coli*. Our results for the intercalating agent 9AA are consistent with those of René et al. (1988) in showing a dose-dependent increase in the frequency of Tc^r colonies in strains whose plasmids contain +1 and −1, but not +2 or −2, frameshift mutations. 9AA induced single-base deletions much more effectively than it induced additions, as has also been found in other assays (Ferguson and Denny, 1990). Both the +1 and −1 frameshifts were measured in a run of GC base pairs at position 536–540 of the *tet* gene, which should be highly susceptible to acridine mutagenesis (Calos and Miller, 1981; Ferguson and Denny, 1990). The pattern of reversion induced by quinacrine, another simple intercalator, was similar to that of 9AA.

The relative revertibility of the frameshift mutations in strains BR871, BR878, and BR879 by the simple acridines and reactive acridines is shown in Table 8. The responses are compared on the basis of revertant frequencies at an equitoxic dosage. The reactive acridines ICR-191, QM, and Entozon are all more potent mutagens in the *tet* assay than the simple acridines. This result is consistent with other studies indicating that the adduct-forming ability of reactive acridines leads to stronger frameshift mutagenesis than does simple intercalation (Brown et al., 1980; Ferguson and Denny, 1991; McCoy et al., 1981). Besides being more potent inducers of −1 and +1 frameshifts, the reactive acridines induced −2 frameshifts at a site of alternating G:C base pairs in strain BR871. Their order of potency in

inducing −2 frameshifts was Entozon > ICR-191 > QM. None of the compounds reverted the −2 frameshift in strain BR872, indicating a lack of +2 mutagenesis, and none were effective inducers of base-pair substitutions in strain TS1800. The revertibility of the base-pair substitution in strain TS1800 by β-propiolactone but not by acridines suggests that this strain reverts only by base-pair substitutions, but the specific substitutions that restore the Tc^r phenotype are not known.

ICR-191, which is a secondary amine and a half mustard, is a more potent frameshift mutagen in the *tet* assay than QM, which is a tertiary amine and a full mustard. Acridine mustards that are secondary amines have similarly been shown to be more mutagenic than tertiary amines in *Salmonella* (DeMarini et al., 1984; Hoffmann et al., 1989), but a comparison of ICR-191 and ICR-170 suggests that the reverse may be true in eukaryotes (Ferguson and Denny, 1991). The strong induction of both +1 and −1 frameshifts by ICR-191 and QM in the *tet* assay is consistent with results for ICR-191 in other assays (Calos and Miller, 1981; Skopek and Hutchinson, 1984). There was a predominance of +1 frameshifts relative to −1 frameshifts, as there was among mutations induced by ICR-191 in a reversion assay in the *lacZ* gene in *E. coli* (Cupples et al., 1990) and in a small sample of mutants induced by ICR-170 in fungi (Burns et al., 1986; Ernst et al., 1985). Nevertheless, the possibility that −1 frameshifts may occur preferentially in other sequence contexts (Calos and Miller, 1981) prevents unequivocal conclusions about the relative effectiveness of acridine mustards in inducing these two classes of frameshifts. It seems clear, however, that the ratio of +1 frameshifts to −1 frameshifts is higher for the acridine mustards than for simple acridines. This same preference is discernible in a comparison of sequences of mutations induced by 9AA and ICR-191 in the *cI* gene of λ in an *E. coli* lysogen (Skopek and Hutchinson, 1984).

The two classes of reactive acridines differed from one another, in that the mustards ICR-191 and QM were highly effective inducers of both +1 and −1 frameshifts, whereas the nitroacridine Entozon resembled the simple acridines in being a relatively weak inducer of +1 frameshifts. However, it was a potent inducer of −1 frameshifts and a stronger inducer of −2 frameshifts than the mustards. Nitroacridines may act either as simple acridines or as reactive acridines depending on the reduction of the nitro group (Ferguson and Denny, 1991). Like other acridines with a nitro group at the 3 position, Entozon should be effectively reduced under physiological conditions (Ferguson and Denny, 1991). Its greater reversion of *hisD3052* than *hisC3076* in *Salmonella* (McCoy et al., 1981) and its difference from 9AA and quinacrine in inducing −2 frameshifts in the *tet* assay are consistent with its acting as a reactive acridine. Unlike ICR-191 and QM, which react with DNA through relatively long mustard side chains, Entozon acts through a reactive intermediate produced by reduction of the nitro group on the ring. The difference in the nature of the covalent adducts may lead to different structural alterations in DNA that cause different mutagenic specificities.

According to a well-known model proposed by Streisinger et al. (1966), frameshift mutations may arise by slipped mispairing in repetitive sequences, especially runs of a single base (e.g., GGGGG). The preferential binding of acridines to DNA bulges associated with slipped mispairing may stabilize these structural alterations and thereby increase the opportunity for frameshift mutations to occur (Ferguson and Denny, 1990). In contrast to slipped mispairing, a nick-processing model has been proposed by Ripley and colleagues to explain acridine-induced mutagenesis in bacteriophage T4; nick processing, which does not require a repetitive sequence, depends on T4 topoisomerase and DNA polymerase (Masurekar et al., 1991; Kaiser and Ripley, 1995). The generality of topoisomerase involvement in acridine mutagenesis remains to be determined. Though slipped mispairing does not encompass all observations on the mutagenicity of acridines, it continues to offer a reasonable model for the induction of −1 and +1 frameshifts in runs of repetitive bases, which is a prominent part of the mutation spectrum of simple acridines and many reactive acridines (Ferguson and Denny, 1990, 1991).

The induction of −2 frameshifts may involve mechanisms that differ from those of +1 and −1 frameshifts. There can be differences in genetic requirements, in that optimal induction by *N*-2-acetylaminofluorene (AAF) of −1 mutations, but not −2 mutations, requires expression of *umuDC*$^+$

and the activated form of the *recA* protein (Janel-Bintz et al., 1994). The −1 frameshifts are readily explained by misalignment in a repetitive sequence when a polymerase is stalled at the site of the adduct (Lambert et al., 1992). The induction of −2 frameshifts may also occur by misalignment or by a mechanism involving AAFs triggering a localized conformational shift from B-DNA to a Z-DNA-like structure that is prone to the −2 mutation (Belguise-Valladier and Fuchs, 1991; Heflich and Neft, 1994; Veaute and Fuchs, 1991). The mutant site in pX_1 has alternating Gs and Cs (Burnouf and Fuchs, 1985) like the *NarI* sequence (GGCGCC) that is a hotspot for the induction of −2 frameshifts by AAF (Koffel-Schwartz et al., 1984). Besides inducing mutations at *NarI* sites in the forward mutation assay, *N*-Aco-AAF, which forms AAF adducts at the C8 position of guanine, is a potent inducer of reversion of a *tet* mutation in a plasmid (pX_2) that, like pX_1, has a CG insert in a *NarI* site (Bintz and Fuchs, 1990). We would speculate that Entozon, another potent inducer of −2 frameshifts in the *tet* reversion assay, may form DNA adducts and cause conformational alterations that resemble those caused by AAF. Unlike acridine mustards, which bind primarily to the N7 position of guanine (Kohn et al., 1987), Entozon may attack other sites, including the C8 position of guanine (McCoy et al., 1981). Moreover, the configuration of the adduct may differ from that of the mustards because of differences in the proximity of the reactive group to the acridine ring.

To evaluate the importance of the permeability mutation of strain ES548/7 in the assay's response to acridines, we tested ICR-191 on the same plasmids in strain AB1157. AB1157 was less sensitive to the mutagenicity and toxicity of ICR-191 than was ES548/7. While the potent induction of +1 and −1 frameshifts was detected in AB1157, albeit at higher dosages, the weaker −2 mutagenicity of ICR-191 was not. Mutations conferring enhanced permeability are therefore important when chemical uptake is a limiting factor in the mutagenic response. Reversion of the T7-resistance mutation can give rise to less permeable bacteria that have a selective advantage in mixed cultures in the presence of toxic chemicals. To avoid an accumulation of nonpermeable cells that reduce the sensitivity of the assay, cultures should routinely be derived from single colonies and tested for genotype, using crystal-violet sensitivity to confirm the presence of the permeability mutation.

Through its use of positive selection, the reversion assay permits the rapid detection of mutagenesis and quickly gives a sense of the relative prevalence of different categories of mutations induced by a mutagen (René et al., 1988). Because of its simplicity, it permits the testing of a greater range of host genotypes (Koffel-Schwartz and Fuchs, 1989; Maenhaut-Michel et al., 1992) than would be practical in the more labor-intensive forward mutation assay in the *tet* gene. The reversion assay also permits the direct screening of chemicals for the ability to induce a specific mutation in a given sequence context (Bintz and Fuchs, 1990) and allows the study of mutations that comprise a small part of the mutation spectrum.

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