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Enhancement of Nitrosoguanidine Mutagenesis by Chloramphenicol in *Escherichia coli* K-12

ROBERT SKLAR

Department of Microbiology, The University of Chicago, Chicago, Illinois 60637

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N-methyl-*N'*-nitro-*N*-nitrosoguanidine-induced mutagenesis in *E. coli* K-12 can be enhanced up to 50-fold by the addition of chloramphenicol with minimal effect on survival. Chloramphenicol does not produce the expected proportionate increase in closely linked double mutations.

N - methyl - *N'* - nitro - *N* - nitrosoguanidine (MNNG) is a powerful mutagen widely used to obtain desired mutant strains. However, the mutagen has the undesirable characteristic of inducing closely linked mutations at a high frequency, which makes the genetic nature of any isolated strain suspect (2). I have found that addition of chloramphenicol, along with the mutagen, results in greatly enhanced mutagenesis, without a comparable increase in linked second-site mutations.

Chloramphenicol enhanced MNNG mutagenesis when reverse mutation or forward mutation was measured in laboratory strain ABAA (F' *thr-1 leu-6 proA2 his-4 thi-1 lacY1 galK2 xyl-5 mtl-1 tsx-33 strA31 sup-37*), an arg$^+$, ara$^+$ P1 derivative (this laboratory) of AB1157, obtained from David Mount. Reversion was measured at the *leu-6* locus. Cells were treated in the M9 minimal medium described by Miller (4). Exponential cells at a density of 2×10^8 to 4×10^8 cells per ml were treated with MNNG in M9 minimal growth medium in the presence or absence of 50 μg of chloramphenicol per ml for 40 min at 37°C. The data of Table 1 illustrate the chloramphenicol enhancement of leucine reversion and nonselected forward mutation to auxotrophy in the same treated population. The magnitude of chloramphenicol enhancement was similar for both types of mutation.

Chloramphenicol greatly enhances mutation induction at MNNG doses greater than 0.1 μg/ml (Fig. 1). Survival after MNNG treatment is only slightly reduced by adding chloramphenicol (Fig. 1). Chloramphenicol treatment does not change the spontaneous mutation rate (3). Chloramphenicol does not enhance mutagenesis by ethyl methane sulfonate (EMS) or UV radiation (Table 1). Exponential cultures were irradiated with 60 J/m^2 of UV light, which resulted in 10% survival. Manipulations of the irradiated culture were done in subdued light. Treatment of cultures with 0.1 M EMS yielded 50% survival. Chloramphenicol did not alter the mutation frequency of *rpoB* or *leu*$^+$ when added along with either mutagen.

In addition to enhancing MNNG mutagenesis, chloramphenicol plus MNNG treatment results in a lower proportion of closely linked double mutations, relative to cultures treated with MNNG alone, as though the chloramphenicol (MNNG) treatment induced only additional single-site mutations. Treated bacterial populations were selected for *rpoB* and screened for second-site mutation at *aceAB* (acetate utiliza-

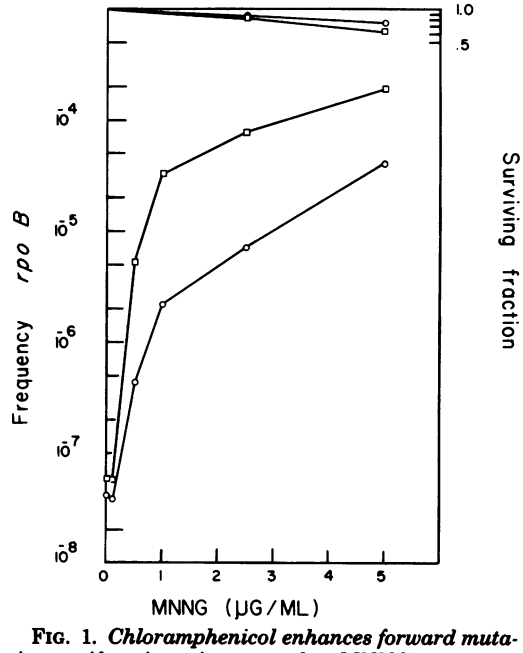

FIG. 1. *Chloramphenicol enhances forward mutation to rifampin resistance at low MNNG concentrations. Cultures were prepared and treated as described in the legend to Table 1, and stationary phase cells were plated on rifampin selection plates and LB plates. Cultures were treated with MNNG* (○) *or MNNG plus 50 μg of chloramphenicol per ml* (□).

TABLE 1. *Effect of chloramphenicol on reversion and forward mutation*[a]

Treatment	Frequency of *leu*$^+$	Frequency of auxotrophy	Frequency of *rpoB*
None	Undetected $<1.0 \times 10^{-8}$	Undetected	4.0×10^{-8}
MNNG (2.5 μg/ml)	2.8×10^{-7}	2.0×10^{-3}	
MNNG (2.5 μg/ml) + chloramphenicol	1.1×10^{-5}	8.0×10^{-2}	
UV (60 J/m^2)	3.6×10^{-7}		4.7×10^{-6}
UV (60 J/m^2) + chloramphenicol	3.4×10^{-7}		3.9×10^{-6}
EMS (0.1 M)	1.4×10^{-6}		1.2×10^{-5}
EMS (0.1 M) + chloramphenicol	1.1×10^{-6}		1.2×10^{-5}

[a] *E. coli* K-12 ABAA was grown at 37°C with aeration to a density of 2×10^8 to 4×10^8 cells per ml in M9 minimal media (2) plus 0.2% Casamino Acids. Cultures were treated with a 2.5-μg/ml dose of MNNG, UV light (60 J/m^2), or 0.1 M EMS for 45 min, with or without chloramphenicol for 40 min (except EMS which was treated for 45 min), and were washed twice with M9 growth medium at 0°C. Cultures exposed to UV light were wrapped in foil to prevent photoreactivation. Cells were resuspended in M9 growth medium and grown with aeration to stationary phase (4 to 6 h). The cultures were then plated on leucine reversion selection plates (4), rifampicin selection plates (4), and LB plates (4). Colonies which appeared on LB plates were replica plated onto minimal plates (4) plus required supplements. Colonies which failed to grow on minimal plates were scored as auxotrophs.

TABLE 2. *Effect of chloramphenicol on closely linked mutation induction by MNNG*[a]

Treatment[b]	Frequency of *rpoB* of *leu*$^+$	Selection	No. of mutants scored	% Mutation at linked locus
MNNG	2.4×10^{-5}	*rpoB*	24/965	2.5[c]
		None	7/996	0.7
MNNG + chloramphenicol	1.3×10^{-4}	*rpoB*	41/804	5.1
		None	32/898	3.6
MNNG	2.8×10^{-7}	*leu*$^+$	3/1,000	0.3[d]
		None	0/2,477	<0.04
MNNG + chloramphenicol	1.1×10^{-5}	*leu*$^+$	11/1,542	0.7
		None	6/1,268	0.5

[a] Closely linked mutation was measured in two genetic systems. In the rifampin system, *rpoB* was selected and mutation at a closely linked marker (*aceAB*) was monitored by replica plating. In system II, *leu-6* was reverted and the revertants were screened by replica plating for the ability to utilize arabinose. Cultures were prepared and treated as described in the legend to Table 1.

[b] Treatment was with a 2.5-μg/ml dose of MNNG with or without 50 μg of chloramphenicol per ml.

[c] Distance between *rpoB* and *aceAB* is 0.9 min (1).

[d] Distance between *leu*-6 and *ara* is 0.4 min.

tion) which is closely linked. In Table 2, system I, the frequency of acetate non-utilization is 3.6-fold higher in populations selected for *rpoB* than in nonselected populations; however, cultures treated with MNNG and chloramphenicol yield a 5.1-fold increase in single *aceAB* mutational events, but only a twofold increase in double mutations. Also in Table 2, system II, MNNG and chloramphenicol treatment increases the frequency of single arabinose utilization events by at least 12.5-fold, but only increases double mutation events by 2.3-fold. Thus, in the rifampin system, the expected percentage of mutation at *aceAB* would be 12.9%, or 104 mutants, whereas only 5%, or 41 mutants, were found; in the leucine system, the expected mutation to arabinose non-utilization in the selected population is 3.8%, or 58 mutants, whereas only 0.7%, or 11 double mutants, were found. It has not been determined whether longer incubations of chloramphenicol plus MNNG treatment will reduce the proportion of closely linked double mutations further.

In conclusion, chloramphenicol plus MNNG treatment increases mutation at single loci without a comparable increase in closely linked double mutations. Addition of rifampin, along with mutagen, also increases mutation at single loci without enhancing double mutations (data not shown). MNNG damage may produce a signal for the induction of an error-free repair system (5) which normally acts to reduce mutational damage. In the absence of this system, single-locus mutation is greatly enhanced.

The ability of chloramphenicol to enhance MNNG-induced single-site mutations 5- to 50-fold without a concomitant proportional increase in closely linked double mutations makes the addition of this drug very helpful in attempts to obtain rare genetic alterations.

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