



Alleviation of type I restriction in adenine methylase (*dam*) mutants of *Escherichia coli*

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Summary. The host-controlled *EcoK*-restriction of unmodified phage λ .O is alleviated in *dam* mutants of *Escherichia coli* by 100- to 300-fold. In addition, the *EcoK* modification activity is substantially decreased in *dam*⁻ strains. We show that type I restriction (*EcoB*, *EcoD* and *EcoK*) is detectably alleviated in *dam* mutants. However, no relief of *EcoRI* restriction (Type II) occurs in *dam*⁻ strains and only a slight effect of *dam* mutation on *EcoP1* restriction (Type III) is observed. We interpret the alleviation of the type I restriction in *dam*⁻ strains to be a consequence of induction of the function which interferes with type I restriction systems.

Key words: DNA restriction – *Dam* mutation – Alleviation of restriction – Bacteriophage λ

Introduction

The *dam* gene of *Escherichia coli* codes for a DNA adenine methylase which methylates within GATC sequences in double stranded DNA (for review see Marinus 1984). The primary phenotype of *dam* mutation is the lack of detectable levels of 6-methyladenine in the DNA of *dam*⁻ strains (Marinus and Morris 1974; Bale et al. 1979). Secondary phenotypes include increased sensitivity to mutagenic agents (e.g. UV irradiation, base analogs, ethyl methanesulfonate), increased spontaneous mutability, increased spontaneous induction of prophages, hyper-recombination phenotype and increased activity of certain transposons (Marinus and Morris 1975; Marinus and Konrad 1976; Glickman et al. 1978; Roberts et al. 1985).

Secondary phenotypes are likely associated with a role for *dam* methylation in the regulation of some systems in cells. For instance, *dam* methylation allows mismatch repair to discriminate between parental and daughter strands and to operate only on newly synthesized strand carrying replication errors (Wagner and Meselson 1976; Pukkila et al. 1983). It ensures that in *dam*⁻ strains a random mismatch correction may inactivate unmethylated mismatch-containing DNA by damaging the DNA in an unknown fashion but which then appears able to induce the expression of some *Dam*⁻ phenotypes associated at least with SOS functions (Peterson et al. 1985; Doutriaux et al. 1986). This idea is supported by the finding that most *Dam*⁻ phenotypes

are suppressed by *mut*(*H*, *L*, *S*) mutations, which render the *dam* cells deficient in mismatch repair (Glickman and Radman 1980; McGraw and Marinus 1980; Lu et al. 1983).

Dam methylation may also modulate directly the expression of some genes containing GATC sites within promoter regions (Braun and Wright 1986; Marinus 1985; Mizusawa et al. 1983; Plasterk et al. 1984; Roberts et al. 1985; Sternberg 1985), and it has been suggested that the *dam* methylation sites may be involved in regulation initiation of chromosomal replication (Messer et al. 1985; Smith et al. 1985).

In this paper we show that one of the properties of *dam*⁻ strains is a drop in the efficiency of type I restriction and modification. Our studies indicate that the restriction alleviation in *dam*⁻ strains is not suppressed by *mut* and *recA* mutations. We suggest that the effect of *dam* mutation on restriction and modification is due to the induction of the function that interferes with the type I restriction systems.

Materials and methods

Bacterial and phage strains. The *E. coli* strains used in this study are listed in Table 1. P1 phage transductions using P1vir were performed as described by Miller (1972). The *dam4* allele was transduced from GM1737 *dam4 cysG*: :Tn5 (Arraj and Marinus 1983) selecting Kan^r colonies and screening for sensitivity to 2-aminopurine. The *dam13*: :Tn9 allele was transduced from GM2159 (Marinus et al. 1983) selecting Cm^r colonies and screening for sensitivity to 2-aminopurine. The *dam*⁻ strains were also tested for sensitivity to *MboI* digestion (Lacks and Greenberg 1977). Strains GM1737 and GM2159 were provided by M.G. Marinus. The donor strains for *mutH*, *mutL* and *mutS* alleles were AB1157 *mutH*: :Tn5, AB1157 *mutL*: :Tn10 and AB1157 *mutS*: :Tn10, respectively (obtained from G.C. Walker). These *mut* alleles were introduced into a *dam*⁻ background by P1 transduction and selection for resistance to kanamycin or tetracycline. These drug-resistant colonies were tested for mutator phenotype and resistance to 2-aminopurine. The *dam*⁻ *mut*⁻ strains obtained were stable in our hands in the presence of antibiotic selection. The strains carrying plasmid NM182 were constructed by mating with *E. coli* 1100 (NM182) (obtained from K. Murray). The strains lysogenic for P1CMts were obtained by chloramphenicol (Cm) selection at 30° C (Miller 1972). BA386 was obtained as a spontaneous Rif^r mutant of TK701. The donor strain

Table 1. *Escherichia coli* strains

Strains	Relevant genotype and/or phenotype	Source or reference
<i>E. coli</i> K12		
TK701	F ⁻ <i>proA2 his-4 thi-1 lacY1 galK2 xyl-1 mtl-1 supE44 r_k⁺ m_k⁺</i>	Kato and Shinoura (1977)
BA386	As TK701 but Rif ^r	This paper
BA835	As BA386 but <i>dam13::Tn9</i>	This paper
BA1029	As BA835 but <i>mutS::Tn10</i>	This paper
BA1031	As BA835 but <i>mutH::Tn5</i>	This paper
BA1078	As BA835 but <i>mutL::Tn10</i>	This paper
BA1089	As BA386 but <i>mutS::Tn10</i>	This paper
BA1090	As BA386 but <i>mutH::Tn5</i>	This paper
BA1091	As BA386 but <i>mutL::Tn10</i>	This paper
BA900	As BA386 but <i>dam4 cysG::Tn5</i>	This paper
BA938	BA386 (NM182 <i>r_{RI}⁺ m_{RI}⁺</i>)	This paper
BA936	BA900 (NM182 <i>r_{RI}⁺ m_{RI}⁺</i>)	This paper
BA908	BA386 (P1CMts)	This paper
BA907	BA900 (P1CMts)	This paper
AB1157	F ⁻ <i>thr-1 leu-6 proA2 his-4 thi-1 argE3 lacY1 galK2 ara14 xyl-5 mtl-1 tsx-33 rpsL31 supE44 r_k⁺ m_k⁺</i>	Bachmann (1972)
BA556	As AB1157 but <i>r_k⁻ m_k⁺ thr⁺</i>	Belogurov et al. (1985)
BA817	As BA556 but <i>dam13::Tn9</i>	This paper
HB129	<i>r_B⁺ m_B⁺</i>	Roulland-Dussoix and Boyer (1969)
BA902	As HB129 but <i>dam13::Tn9</i>	This paper
BZ216	<i>r_D⁺ m_D⁺</i>	T.A. Bickle
BA905	As BZ216 but <i>dam4 cysG::Tn5</i>	This paper
<i>E. coli</i> C		
C	<i>r_o m_o</i>	Bertani and Weigle (1953)
BA819	As <i>E. coli</i> C but <i>dam13::Tn9</i>	This paper

for *recA::Cm^r*, DB1317 was provided by K.F. Wertman. The strain DM49 *lexA3* was obtained from D. Mount.

The bacteriophage referred to as λ was λ vir (obtained from R. Devoret). Unmodified phages, denoted by λ .O were grown on *E. coli* C *r_o m_o*, which lacks restriction and modification functions. Modified phages denoted by λ .K were grown on the *r_k⁺ m_k⁺* strain AB1157. Phage λ b506cI-857bio256(*ral*⁻) was obtained from K. Murray.

Media. L broth contained 10 g tryptone, 5 g yeast extract, 5 g NaCl per liter of water, pH 7.2. T agar (1% tryptone, 0.5% NaCl) was used at 1.2% for bottom agar and 0.6% for top agar in experiments with phages. When used, supplements were added at the following concentrations: 400 μ g/ml 2-aminopurine, 20 μ g/ml chloramphenicol, 40 μ g/ml kanamycin, 100 μ g/ml rifampicin (Rif), 20 μ g/ml tetracycline, 100 μ g/ml streptomycin.

Results and discussion

EcoK restriction is alleviated in *dam*⁻ strains

We have tested type I restriction in *E. coli* *dam*⁺ and *dam*⁻ strains by measuring the efficiency of plating of unmodified

Table 2. Effect of *dam* mutations on *EcoK* restriction

Restricting strains	Efficiency of test phage plating ^a	Relief of restriction ^b
BA386 parent strain	1 × 10 ⁻⁴	1
BA900 <i>dam4</i>	3 × 10 ⁻²	300
BA835 <i>dam13</i>	1 × 10 ⁻²	100
BA1029 <i>dam13 mutS</i>	3 × 10 ⁻²	300
BA1031 <i>dam13 mutH</i>	2 × 10 ⁻²	200
BA1078 <i>dam13 mutL</i>	2 × 10 ⁻²	200
BA1089 <i>mutS</i>	5 × 10 ⁻⁴	5
BA1090 <i>mutH</i>	5 × 10 ⁻⁴	5
BA1091 <i>mutL</i>	5 × 10 ⁻⁴	5

^a The efficiency of plating of unmodified test phage λ .O was determined by measuring the ratio of phage titer on the restricting strains to the titer on the non-restricting strain AB1157 *r_k⁺ m_k⁺* (BA556); in all cases the plating efficiency of modified phage λ was equal to 1

^b The relief of *EcoK* restriction is the efficiency of plating of λ .O on mutants relative to that on the parent strain BA386

phages λ .O. The experiments described in Table 2 show that *EcoK* restriction of phage λ .O is alleviated in *dam*⁻ strains by 100- to 300-fold. Similar results were obtained with other unmodified test phage, T7ocr⁻.O (data not shown) which is sensitive to *EcoK* restriction (Studier 1975). These findings suggest that *EcoK* restriction endonuclease is detectably inhibited in *dam* mutants compared to a *dam*⁺ background. It should be noted that the extent of the restriction alleviation in *dam*⁻ strains depends on the genetic background of the strains used. For example, in *dam*⁻ derivatives of AB1157 and TK701, relief of restriction is larger than in *dam* mutants of NR3752 (data not shown).

The *dam* mutations are known to be highly pleiotropic, and most *Dam*⁻ phenotypes are suppressed by mutations in genes *mutH*, *mutL* and *mutS* (McGraw and Marinus 1980; Glickman and Radman 1980). However, the presence of the second site mutations *mutH*, *mutL* and *mutS* in a *dam*⁻ background does not suppress the restriction alleviation (Table 2). Moreover, *EcoK* restriction is slightly alleviated by these *mut* mutations in a *dam*⁺ background (Table 2).

The expression of some genes of the SOS regulon is known to be increased in *dam*⁻ strains (Peterson et al. 1985). Therefore we were interested in the possibility that the restriction alleviation in *dam*⁻ strains might result in the induction of SOS functions. Since mutants *dam*⁻ *recA*⁻ and *dam*⁻ *lexA*⁻ are inviable (McGraw and Marinus 1980), the viable combinations *dam4 mutS::Tn10 recA::Cm^r* and *dam13 mutS::Tn10 lexA3 (Ind⁻)* were constructed. However, we found that mutations *recA* and *lexA* had no effect on restriction alleviation in *dam*⁻ *mut*⁻ backgrounds (data not shown), and this finding suggests that the restriction alleviation in *dam*⁻ strains is not an SOS function.

Effect of *dam* mutations on the different restriction systems in *E. coli*

We have studied the effect of *dam* mutations on the different restriction systems in *E. coli*. Data in Table 3 show that type I restriction (*EcoB*, *EcoD*, *EcoK*) is detectably alleviated in *dam* mutants. However, no relief of *EcoRI* restriction (Type II) occurs in *dam*⁻ strains and only a slight

Table 3. Effect of *dam* mutations on different *Escherichia coli* restriction systems

Restriction system ^a	Efficiency of test phage plating on strains ^b		Relief of restriction ^c (<i>dam</i> ⁻ / <i>dam</i> ⁺)
	<i>dam</i> ⁺	<i>dam</i> ⁻	
1. <i>EcoK</i> (Type I)	1 × 10 ⁻⁴	1–3 × 10 ⁻²	100–300
2. <i>EcoB</i> (Type I)	1 × 10 ⁻⁴	1 × 10 ⁻³	10
3. <i>EcoD</i> (Type I)	2 × 10 ⁻⁴	4 × 10 ⁻³	20
4. <i>EcoRI</i> (Type II)	2 × 10 ⁻⁴	2 × 10 ⁻⁴	1
5. <i>EcoP1</i> (Type III)	5 × 10 ⁻⁵	6 × 10 ⁻⁴	12

^a The restricting strains used were: 1, BA386 *dam*⁺, BA835 *dam13* or BA900 *dam4*; 2, HB129 *dam*⁺, BA902 *dam13*; 3, BZ216 *dam*⁺, BA905 *dam4*; 4, BA938 *dam*⁺ (NM182), BA936 *dam4* (NM182); 5, BA908 *dam*⁺ (P1), BA907 *dam4* (P1). Since strains 4 and 5 contain 2 restriction systems, we used λ .K test phages in order to subject them only to the *EcoRI* and *EcoP1* restriction systems.

In experiments with strains 1, 2 and 3 we used λ .O test phage

^b The efficiency of plating of unmodified test phages was determined by measuring the ratio of phage titer on the restricting strains relative to that on the non-restricting strain AB1157 *r*_k⁻ *m*_k⁺ (BA556); in all cases the plating efficiency of modified phage λ was 1

^c The relief of restriction is the efficiency of plating of unmodified test phages on *dam*⁻ strains relative to that on a *dam*⁺ background

effect of *dam* mutation on *EcoP1* restriction (Type III) is observed.

It should be noted that the effect of *dam* mutations on *EcoP1* restriction may be indirect and reflect the relative instability of P1 prophage in a *dam*⁻ host in which, as indicated above, the frequencies of mutation and some transposition events are increased. It is known that P1 lysogenicity is not fully stable even in wild-type cells and many mutants of P1 prophage affected in different phage functions (including the restriction function) arise mainly as a result of transposition events (Arber et al. 1980; Sengstag and Arber 1983; W. Arber, personal communication). Therefore, the possibility, that the effect of *dam* mutation on *EcoP1* restriction is due to the increase in frequency of spontaneous *r*_{P1}[±] *m*_{P1}[±] mutants of P1 prophage in *dam*⁻ strains can be imagined. Another possibility is that *dam* mutation influences expression of *hsd* or other genes of P1 prophage and thus affects *EcoP1* restriction. In both cases, the mechanism of restriction alleviation will be specific to the type III restriction. It is possible therefore that the mechanisms of alleviation of the types I and III restriction in *dam*⁻ strains may be essentially different.

EcoK modification is decreased in *dam*⁻ strains

The alleviation of type I restriction in *dam*⁻ strains could be explained solely by the induction of the function which controls a new type of DNA modification or somehow stimulates host-controlled modification. However, we did not find evidence for this assumption.

Firstly, the presence of *dam* mutations in the host strain *r*_k⁻ *m*_k⁻ has no effect on *EcoK* modification of λ .O progeny (data not shown), suggesting that the restriction alleviation in *dam*⁻ strains is not due to an induced DNA modifying activity.

Secondly, the experiments described in Table 4 show that the efficiency of host-controlled *EcoK* modification

Table 4. Effect of *dam* mutation on progeny phage modification in *r*_k⁻ *m*_k⁺ strains^a

Phages	Efficiency of modification of progeny phages, grown in the strains ^b		
	1. <i>dam</i> ⁺	2. <i>dam13</i>	<i>dam13/dam</i> ⁺
<i>λ</i> bio256(<i>ral</i> ⁻).O	0.02	0.001	0.05
<i>λ</i> (<i>ral</i> ⁺).O	1.0	0.1	0.1

^a Unmodified phages were grown for one cycle in *r*_k⁻ *m*_k⁺ strains, and the efficiency of progeny phage modification was determined as the ratio of the phage titer on the restricting indicator strain *r*_k⁺ to the phage titer on the non-restricting indicator strain *r*_k⁻ (Zabeau et al. 1980). The strains were grown in L broth to about 2 × 10⁸ cells/ml, harvested and resuspended at a concentration of 1 × 10⁹ cells/ml. Phages were adsorbed at a ratio of 0.01 phages/cell for 20 min at 37° C. The infected cells were diluted 1000-fold in fresh L broth and incubated without aeration for 90 min at 37° C. The progeny phages obtained were plated on isogenic *r*_k⁺ and *r*_k⁻ strains (AB1157 and BA556)

^b The strains used were: 1, BA556 *dam*⁺ *r*_k⁻ *m*_k⁺; 2, BA817 *dam13* *r*_k⁻ *m*_k⁺

of λ .O phages is 10-fold decreased in a *dam*⁻ background compared to a *dam*⁺ background.

Taken together, data in Tables 2 and 4 show that both *EcoK* restriction and *EcoK* modification are reduced in *dam*⁻ strains. The simple interpretation of these results is that the alleviation of type I restriction in *dam*⁻ strains is due to induction of the function which interferes with type I restriction systems and affects DNA cleavage and methylation. This suggestion is consistent with the observations that *Dam* methylation plays a pivotal role in the coordination of some cellular processes and modulates expression of some genes in *E. coli* (Mizusawa et al. 1983; Braun and Wright 1986; Marinus 1985; Roberts et al. 1985; Sternberg 1985). It is also possible that *Dam* methylation influences directly expression of the *hsd* genes that code for the host-dependent restriction systems.

It should be noted that alleviation of *EcoK* restriction has also been observed in *E. coli* cells following UV irradiation (Day 1977). This phenomenon has been termed UV-induced restriction alleviation (UV-alleviation). It will be interesting to compare this effect and the restriction alleviation, induced in *dam*⁻ strains.

The observation that UV-alleviation does not occur in *recA* and *lexA* mutants (Day 1977) argues strongly that UV-alleviation, in contrast to the alleviation induced in *dam*⁻ strains, is one of the SOS functions (Little and Mount 1982; Walker 1984). On the other hand, both effects seem to be specific for *EcoK* restriction (Type I), which is alleviated in both cases by 100-fold (Table 3; Toms and Wackernagel 1982). It is not clear what the relation is between these two types of restriction alleviation. It is possible, however, that there are two alternative pathways with different signals (unmethylated DNA and damaged DNA) for the induction of one type of restriction alleviation function in *E. coli* cells.

For further studies we have isolated and mapped mutations which suppressed the restriction alleviation in *dam*⁻ strains (manuscript in preparation).

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