https://bjm.ui.ac.ir/?lang=en

Journal of Microbial Biology
E-ISSN: 3060-7647
14rd Year, Vol. 14, No. 55, 2025 pp. 1-6
Received: 15/08/2024 Accepted: 16/11/2024

(Research Paper)

DinI could be a suitable option in drug targeting strategies to reduce SOS mutagenesis

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Abstract:
Ciprofloxacin induces the SOS response. Induction of the SOS response confers resistance to ciprofloxacin through activation of DNA polymerase V. However, mutations in *lexA*, *umuDC* and especially *recA* prevent the evolution of resistance to ciprofloxacin. Alternatively, mutations in SOS proteins that regulate RecA activity, such as DinI, can reduce SOS mutagenesis. The aim of this study was to investigate the effect of DinI inactivation on the frequency of mutagenesis and expression of the *umuD* and *rdgC* genes after treatment with ciprofloxacin and to examine the promoter region and the 3′ end of the *recA* gene for possible changes in *dinI* (SM1 and SM2) mutants. Ciprofloxacin-resistant clones (SM1 and SM2) derived from the JW10481 (*dinI*$^{-}$) strain were used in this study. Mutagenesis analysis and real-time PCR were used to measure the frequency of mutant cells and the expression of *umuD* and *rdgC* genes in mutants, respectively. The promoter region and *recA* gene sequence were examined by PCR amplification and DNA sequencing in *dinI*$^{-}$ clones. SOS mutagenesis was significantly reduced ($P<0.05$) in SM1 and SM2 clones**.** These clones (*dinI*$^{-}$) did not show overexpression of the *umuD* gene. *rdgC* gene was overexpressed in SM2, but not in the M2 mutant with intact *dinI* gene. Furthermore, DNA sequencing did not reveal any change in the *recA* gene sequence. The low frequency of mutagenesis and *umuD* expression in clones lacking DinI protein activity demonstrated the importance of this protein in SOS mutagenesis. In conclusion, DinI may be a suitable option in drug targeting strategies to enhance the efficacy of ciprofloxacin in combination therapy against a variety of infections caused by *Escherichia coli* and other Gram-negative bacteria with homologues of this protein.
Keywords: Ciprofloxacin, DinI, *Escherichia coli*, Mutagenesis, SOS response

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Pourahmad Jaktaji R & Mohammadi S. DinI could be a suitable option in drug targeting strategies to reduce SOS mutagenesis. *Journal of Microbial Biology*, 2025; 14 (55): 1-6. https://doi.org/10.22108/bjm.2024.142477.1610

Introduction

Ciprofloxacin (CIP) is a potent antibiotic that targets DNA gyrase in bacteria. However, resistance to CIP develops in *Escherichia coli* (*E. coli*) due to mutations in target genes, mainly *gyrA*, and overactivation of the AcrAB-TolC efflux pump. The development of CIP resistance requires overactivation of the SOS response following exposure to CIP by interfering with gyrase activity and inhibiting replication fork progression. The stalled replication forks produce suitable substrates for RecA (1-3). Upon binding of RecA protein to single-stranded DNA (ssDNA) and formation of a cofilament, RecA either mediates strand exchange or promotes LexA autocleavage, leading to the production of many SOS proteins, including RecA and DinI (4). In addition to ssDNA and LexA, other proteins including DinI, UmuD and RecX bind to the helical groove of RecA filaments (5-6).

DinI, RecX, RecFOR and RdgC have regulatory effects on RecA activity. RecX promotes filament disassembly, whereas DinI stabilizes RecA filaments after their formation (7-8). The RecFOR complex induces both association and dissociation of the RecA filament, and RdgC interferes with RecA activities (9). RdgC is an essential factor for growth in *priA* mutants as it counteracts RecFOR-mediated RecA loading on ssDNA (10).

Given the importance of SOS induction in causing mutagenicity and antibiotic resistance, finding a target to reduce SOS induction would be very beneficial. Inactivation of the DinI regulatory protein would be one way to reduce RecA activity and SOS mutagenesis following CIP treatment. To date, many chemicals have been proposed to inhibit RecA and LexA activities in *Escherichia coli* (11-12), but the effect of DinI inactivation on reducing SOS mutagenesis has not been investigated. In the previous study, we generated CIP resistant clones (SM1 and SM2) from the strain JW10481 (*dinI*$^-$) strain and measured their resistance to CIP and the expression of the *recA* gene in them. The SM2 clone (*dinI*$^-$) overexpressed *recA*, but 1.6 times less than the C17 mutant (*dinI*$^+$) with the same level of resistance to CIP (13). In the present study, we measured the frequency of mutagenesis and the expression of *umuD* and *rdgC* genes in SM1 and SM2 clones and found that inactivation of DinI inhibits SOS mutagenesis. It has been proposed that the C-terminal part of RecA modulates RecA binding to different substrates, including ssDNA, LexA, DinI and UmuD. Therefore, the promoter region (containing the SOS box) and the 3′ end of the *recA* gene were examined for possible alterations in the above clones. It was found that the reduction in SOS mutagenesis was not related to the alteration of the *recA* SOS box or its 3ˈend sequence. These results suggest that one way to reduce SOS mutagenesis is to inactivate the DinI protein.

Materials and Methods

Strains and mutants: CIP was purchased from Sigma-Aldrich Company. MG1655 wild type strain (carrying intact *dinI* gene, sensitive to CIP, 0.008 µg/ml), *dinI*$^-$ clones SM1 (sensitive to CIP, 0.3 µg/ml) and SM2 (moderate resistant to CIP, 1 µg/ml), both derived from JW10481 (BW25113 *ΔdinI*::Kan^r, Keio collection) (13-14), W49 (*gyrA* mutant, sensitive to CIP, 0.6 µg/ml), C17 (moderate resistant to CIP, 1 µg/ml) and M3 (highly resistant to CIP, 40 µg/ml) mutants, both carrying the *gyrA marOR* double mutation (15), were used in this study.

CIP-induced mutagenesis: CIP-induced rifampin resistance testing was performed according to a previous study (16). Fresh cultures of MG1655, two *dinI*$^-$ clones and M3 in LB without and with CIP (0.1 ^{x} MIC of each strain) were placed in a shaking incubator at 37 °C for 4 h, then transferred to fresh LB broth and incubated overnight under the above conditions. Total cells were counted on LB agar with and without rifampin (20 µg/ml) to calculate the frequency of rifampin resistant mutants. The experiment was performed three times.

Quantitative reverse transcription PCR (qRT-PCR): The qRT-PCR assay has been described previously (15). Fresh cultures of six strains and mutants were used to inoculate LB broth containing CIP (0.1 MIC of each strain). The cultures were incubated at 37 °C to reach an OD value of 0.4. The cultures were then used to extract total RNA using an RNA extraction kit (Qiagen, USA). Total RNA was used to synthesize cDNA (Reverse Transcriptase kit, Fermentas) for qRT-PCR using a SYBR Green kit (Yektatajhiz, Iran) and the micPCR machine (Biomolecular System,

Australia). The primers are listed in Table 1. The thermal cycling condition was 95 °C for 5 min followed by 40 cycles of denaturation (95 °C, 10 s), annealing (50 °C–55 °C for 15 s, depending on the primer pairs used) and extension (72 °C for 20 s). Expression data are the mean of two assays.

Statistical analysis: SPSS version 16 software student's t-test was used to calculate significant differences between expression data.

Amplification and sequencing of the *recA* gene: A single colony of each clone (SM1 and SM2) on LB agar containing CIP (0.1 MIC of each mutant) was used as a source of DNA template. Two pairs of primers were used to amplify the *recA* gene (Table 1) as previously described (15). The thermal cycling condition was 95 °C for 3 min, followed by 30 cycles of denaturation (95 °C for 45 s), annealing (52 °C for 30 s) and extention (72 °C for 1 min). Final extention for 4 min. The sequence of the amplified products was determined using both primers. The nucleotide sequences were compared with that of strain MG1655 (ID: 947170) using the multiple alignment tool (Clustal W). The sequence of the SOS box upstream of the *recA* gene was obtained from previous work (17).

Table 1. Primers used for real-time PCR and colony PCR

Gene	Primer sequence (5'-3')	Amplicon size (bp)	Refernce
dinI	AGTATGCGTTTCCTGATAATGAA TATTCGCTGACAAACCAGTCA	157	This work
umuD	GATCCAGCATTCCAGC CCGTAAACTCGCCGTC	155	Pourahmad and Pasand, 2016
rdgC	ACCATTGGCAACGTGC CGTGCAGAAGAGATGG	132	This work
gapA	ACTTACGAGCAGATCAAAGC AGTTTCACGAAGTTGTCGTT	170	Pourahmad and Pasand, 2016
recA[a]	CAGCGGCGACCGTGA CAGCAGCGTGTTGGACT	734	Pourahmad and Pasand, 2016
recA[b]	AGCCAGGCGATGCGT GCCGCAGATGCGACC	585	Pourahmad and Pasand, 2016

[a&b] primers for amplification of the SOS box and 3'end of *recA* gene, respectively.

Results

The amount of mutagenesis was significantly decreased in SM1 (0.6 ratio Rif^R/total cell) and SM2 (0.5 ratio Rif^R/total cell) compared to the wild type strain (6 ratio Rif^R/total cell, $P<0.05$) in the presence of CIP (Fig. 1). The same results were obtained in the absence of CIP. The mutagenesis value for SM1 and SM2 with different levels of resistance to CIP were not significantly different ($P<0.05$), but were significantly lower than the mutagenesis value for M3 (70 ratio Rif^R/total cell) with high levels of resistance to CIP in the presence of CIP (Fig. 1). Similar results were observed in the absence of antibiotic. Moreover, the levels of mutagenesis in MG1655 and M3 were significantly higher in the presence of antibiotic than in the absence of CIP (Fig. 1).

The quantity and quality of the RNA samples were measured by spectrophotometry. After checking that the RNA samples were not contaminated with DNA, they were used to synthesize cDNAs. cDNAs used to measure gene expression. None of the *dinI*$^-$ mutants (SM1 and SM2) showed overexpression of the *umuD* gene (Table 2). These results are in agreement with the mutagenesis results. Our results showed that the *dinI*$^+$ mutants (C17 and M3) were able to overexpress *dinI* gene (Table 2). *rdgC* gene overexpressed in the SM2 mutant but not in SM1 (*dinI*$^-$ mutant) and C17 and M3 (*dinI*$^+$ mutants) (Table 2).

After evaluating the quality of the RecA PCR products by gel electrophoresis, their sequences were determined by DNA sequencing. The sequences were visualised using Chromas version 2.6.6 (Fig. 2). Comparison of the FASTA format of the sequences with that of MG1655 using Clustal W showed no change in the sequence of the *recA* gene and its upstream region in SM1 and SM2.

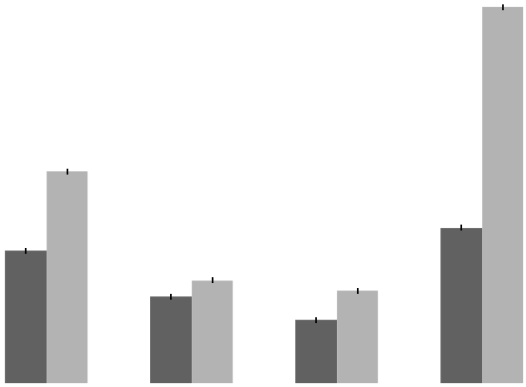
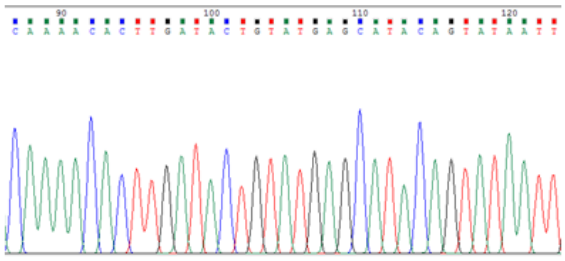


Fig. 1. Frequency of rifampin resistant (Rif^R) mutants. The proportion of Rif^R cells were either untreated (black bar) or treated with CIP (grey bar). Lanes 1, 2, 3 and 4 are wild type, SM1, SM2 and M3. Values are means of three independent experiments ±SD.

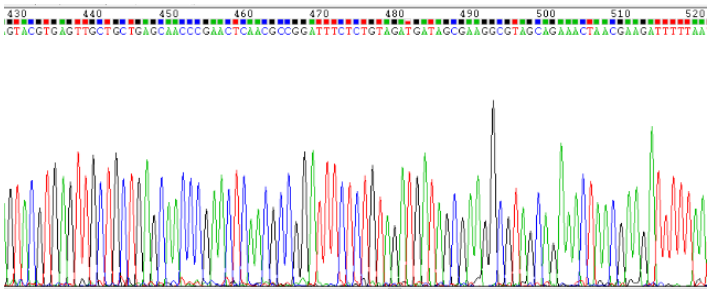
Table 2. Relative expression of genes.

Strain/mutant	*rdgC*	*dinI*	*umuD*
MG1655	1	1	1
W49	1	0.7	-
C17	0.9	2.1	-
M3	1.3	3.54	-
SM1	1.8	-	0.9
SM2	2.03	-	1.82

In all cases, the standard deviation was less than 10% of the mean.



T A C TG T A T GA G C A T A C A G TA T



GTA CGT GAG TTG CTG CTG AGC AAC CCG AAC TCA ACG CCG GAT TTC TCT GTA GAT GAT AGC GAA GGC GTA GCA GAA ACT AAC GAA GAT TTT TAA

Val Arg Glu Leu Leu Leu Ser Asn Pro Asn Ser Thr Pro Asp Phe Ser Val Asp Asp Ser Glu Gly Val Ala Glu Thr Asn Glu Asp Phe *****

Fig. 2. Partial upstream (**A**) and downstream (**B**) DNA sequence of the *recA* gene. **A**. The sequence of the SOS box is underlined and bolded. **B**. The end of *recA* gene and its deduced amino acid sequence are shown.

https://doi.org/10.22108/bjm.2024.142477.1610

Discussion and Conclusion

The effect of DinI inactivation on the expression of *umuD* and *rdgC* genes in *dinI*$^{-}$ clones SM1 and SM2 was investigated. Our results showed that *rdgC* was overexpressed but *umuD* was not in these clones. In a previous study, *recA* was found to be overexpressed in mutants (with intact *dinI* gene and moderate resistance to CIP) (15). It was also found that the *recA* gene was overexpressed in the SM2 mutant (without intact *dinI* gene and intermediate level of resistance to CIP; however, the overexpression was less significant than that seen in the mutant with active *dinI* gene ($P<0.05$) (13). DNA sequencing results showed that this decrease in *recA* expression was not associated with changes in the upstream region of the *recA* gene containing the SOS box.

The acquisition of high levels of resistance requires more SOS signals through efficient cleavage of LexA and UmuD. Our finding that *umuD* was not overexpressed in *dinI* mutants indirectly implies that the mutation frequency in *dinI* mutants is low. This is also confirmed by our finding that the mutation frequency in SM1 and SM2 was low in the presence and absence of CIP. This finding also showed that CIP can enhance SOS mutagenesis in the presence of intact DinI protein.

On the other hand, the RdgC protein inhibits recombination and SOS induction (18-19). RdgC competes with RecA for binding to duplex DNA. DinI enhances the ability of RecA to compete with RdgC (19). Therefore, it was possible that in CIP treated cells lacking DinI, RecA cannot properly compete with RdgC for binding to duplex DNA and therefore cannot generate sufficient SOS signals. This possibility was indirectly revealed by the finding that the *rdgC* gene was over-activated in the *dinI*$^{-}$ mutant (SM2), but not in the *dinI*$^{+}$ mutant (M3). In addition, the lower expression of *recA* and *umuD* suggests that the RecA loading activity of RecF may be reduced in the *dinI* mutant following *rdgC* overexpression. This possibility is consistent with previous finding that showed RdgC interferes with loading of RecA via RecFOR on ssDNA (10). Moreover, our previous finding showed overexpression of *recF* in SM1 (20) and *dinI*$^{+}$ mutants following exposure to CIP (unpublished data). Again, this finding suggests that a factor may prevent RecF activity. The effect of RdgC protein on RecF in the absence of DinI requires further investigation.

Moreover, the *dinI* gene was overexpressed in *dinI*$^{+}$ mutants (C17 and M3). Therefore, it was expected that in the presence of DinI, RecA could adequately compete with RdgC for binding and forming stabilized filament on DNA. Taken together, there might be an inverse relationship between the expression and activity of RdgC and DinI.

Additionally, our results indicated that the 3′ end of RecA remained unchanged in SM1 and SM2 clones. Therefore, we did not expect any change in the binding affinity of RecA to ssDNA, LexA, DinI and UmuD. Furthermore, the upstream sequence of the *recA* gene is intact in SM1 and SM2. Thus, the lower expression of *recA* in SM1 and SM2 is not related to an alteration in the SOS box.

In this study, the *dinI* mutants SM1 and SM2 were used to evaluate the importance of the DinI protein in SOS mutagenesis. These mutants had a low frequency of mutagenesis and *umuD* expression. Therefore, it is concluded that in addition to RecA and LexA, DinI may be a suitable target for the design of an adjuvant to enhance the efficacy of CIP against *E. coli* and other Gram-negative genera harboring DinI homologues.

Acknowledgments

We acknowledge Shahrekord University for financial support and Biotechnology Center at Shahrekord University to provide real-time PCR machine.

Declaration of interest statement

The authors declare that they have no conflict of interest.

Data availability statement

All data generated or analyzed during this study are included in this published article.

References

(1) Revitt-Mills SA., Wright EK., Vereker M., O'Flaherty C., McPherson F., Dawson C., et al. Defects in DNA double-strand break repair re-sensitise antibiotic-resistant *Escherichia coli* to multiple bactericidal antibiotics. *Microbiology Open* 2022; 11(5): e1316. https://doi.org/10.1002%2Fmbo3.1316

(2) Amarh V., Arthur PK. DNA double strand break formation and repair as targets for novel antibiotic combination chemotherapy. *Future Science* 2019; 5(8): FSO411. https://doi.org/10.2144%2Ffsoa-2019-0034

(3) Baharoglu, Z., Mazel, D. SOS, the formidable strategy of bacteria against aggressions. *FEMS Microbiology Review* 2014; 38: 1126-1145. https://doi.org/10.1111/1574-6976.12077

(4) Maslowska KH., Makiela-Dzbenska K., Fijalkowska IJ. The SOS system: A complex and tightly regulated response to DNA damage. *Environ Mol Mutagen* 2019; 60(4): 368-384. https://doi.org/10.1002/em.22267

(5) Alekseev A., Pobegalov G., Morozova N., Vedyaykin A., Cherevatenko G., Yakimov A., et al. A new insight into RecA filament regulation by RecX from the analysis of conformation-specific interactions. *ELife* 2022; 11: e78409. https://doi.org/10.7554/eLife.78409

(6) Gao B., Su L., Won A., Zhou C., Feng Y. Structural basis for regulation of SOS response in bacteria. *PNAS* 2023; 120(2): 1-9. https://doi.org/10.1073/pnas.2217493120

(7) Lusetti SL., Voloshin ON., Inman RB. The DinI protein stabilizes RecA protein filaments. *Journal of Biological Chem*istry 2004; 279(29): 30037-30046. https://doi.org/10.1074/jbc.M403064200

(8) Ragone S., Maman J.D., Furnham N., Pellegrini L. Structural basis for inhibition of homologous recombination by the RecX protein. *EMBO J*ournal 2008; 27(16): 2259-2269. https://doi.org/10.1038%2Femboj.2008.145

(9) Briggs GS., Yu J., Mahdi AA., Lloyd RG. The RdgC protein employs a novel mechanism involving a finger domain to bind to circular DNA. *Nucleic Acid Res*earch 2010; 38(19): 6433-6446. https://doi.org/10.1093%2Fnar%2Fgkq509

(10) Moore T., McGlynn P., Ngo HP., Sharples GJ., Lloyd RG. The RdgC protein of *Escherichia coli* binds DNA and counters a toxic effect of RecFOR in strains lacking the replication restart protein PriA. *EMBO J*ournal 2003; 22(3): 735-745.https://doi.org/10.1093%2Femboj%2Fcdg048

(11) Mo CY., Culyba MJ., Selwood T., Kubiak JM., Hostetler ZM., Jurewicz AJ, et al. Inhibitors of LexA autoproteolysis and the bacterial SOS response discovered by an academic-industry partnership. *ACS Infection Dis*ease 2018; 4(3): 349-359. https://doi.org/10.1021/acsinfecdis.7b00122

(12) Alam Md K., Alhamzi A., DeCoteau JF., Luo Y., Geyer C. R. RecA inhibitors potentiate antibiotic activity and block evolution of antibiotic resistance. *Cell Chemical Biol*ogy 2016; 23(3): 381-391. https://doi.org/10.1016/j.chembiol.2016.02.010

(13) Mohammadi S., Pourahmad R., Mahzonieh M. R. *recA* gene expression in ciprofloxacin resistant *Escherichia coli dinI* mutant. *Journal of Microbial Biology* 2018; 7(26): 65-72. https://doi.org/10.22108/bjm.2017.102689.1034 [In Persian].

(14) Baba T., Ara T., Hasegawa M., Takai Y., Okumura Y., Baba M., et al. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Molecular System Biol*ogy 2006; 2, 2006-2008. https://doi.org/10.1038/msb4100050

(15) Pourahmad Jaktaji R., Pasand S. Overexpression of SOS genes in ciprofloxacin resistant *Escherichia coli* mutants. *Gene* 2016; 576(1): 115-118. https://doi.org/10.1016/j.gene.2015.09.069

(16) Pourahmad Jaktaji R., Nourbakhsh Rezaei SM. Inactivation of UmuC protein significantly reduces resistance to ciprofloxacin and SOS mutagenesis in *Escherichia coli* mutants harboring intact *umuD* gene. *Jundishapur Journal of Microbiol*ogy 2020; 13(12): e111828. https://doi.org/10.5812/jjm.111828

(17) Galkin VE., Britt RL., Bane LB., Yu X., Cox MM., Egelman EH. Two modes of binding of DinI to RecA filament provide a new insight into regulation of SOS response by DinI protein. *Journal of Molecular Biol*ogy 2011; 408(5): 815-824. https://doi.org/10.1016/j.jmb.2011.03.046

(18) Tessmer I., Moore T., Lloyd RG., Wilson A., Erie DA., Allen S., et al. AFM studies on the role of the protein RdgC in bacterial DNA recombination. *Journal of Molecular Biol*ogy 2005; 350(2): 254-262. https://doi.org/10.1016/j.jmb.2005.04.043

(19) Drees JC., Chitteni-Pattu S., McCaslin DR., Inman RB., Cox MM. Inhibition of RecA protein function by the RdgC protein from *Escherichia coli*. *Journal of Biological Chemistry* 2006; 281(8): 4708-4717. https://doi.org/10.1074/jbc.M513592200

(20) Moosavi MS., Pourahmad R., Mahzoonieh MR. Expression of RecF and GyrB genes in a low ciprofloxacin-resistant *Escherichia coli* mutant. *Journal of Microbial Biology* 2018; 7(27): 53-60. https://doi.org/10.22108/bjm.2018.109936.1115 [In Persian].