

A mutant LexA repressor harboring a cleavage motif cysteine-glycine remains inducible

Michèle Granger-Schnarr, Pascale Oertel and Manfred Schnarr

Institut de Biologie Moléculaire et Cellulaire, CNRS and Université Louis Pasteur, 15, rue Descartes, 67084 Strasbourg Cédex, France

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Using site-directed mutagenesis of the *lexA* gene we have changed the amino acid Ala-84 of the LexA repressor for a cysteine. The reason for this change was the striking homology between LexA and UmuD and the comparable size of the two amino acid side chains. Using an in vivo repression/induction assay it is shown that the LexA-Cys-84 mutant remains inducible by mitomycin C and UV irradiation essentially in the same way as the wild-type repressor.

LexA repressor; UmuD protein; SOS induction; Genotoxic agent; Site-directed mutagenesis

1. INTRODUCTION

The LexA repressor of *Escherichia coli* regulates the expression of about twenty different 'SOS' genes involved mainly in DNA repair, SOS mutagenesis, cell division and the production of bacterial toxins [1,2]. In the repressed state the protein binds to its target sequences (SOS operators) via its amino-terminal domain [3] hindering, at least in the case of the *uvrA* gene, the RNA polymerase to bind to the promoter [4]. The contacts between a typical SOS operator and LexA are reminiscent of those found for 'helix-turn-helix' DNA binding proteins [5] but so far it has not been proved that LexA contains this wide-spread DNA binding motif.

Upon UV irradiation or treatment with chemical carcinogens LexA inactivates itself by proteolysis in a reaction which involves an 'activated' form of the RecA protein. This specific cleavage takes place between amino acids Ala-84 and Gly-85 of LexA producing two fragments, an amino-terminal one that is always able to bind specifically

operator DNA [3] and a carboxy-terminal one that dimerizes essentially as the entire protein (Schnarr, M. et al., submitted). The same dipeptide is found within the cleavage site of the SOS inducible repressors of the bacteriophages lambda, 434 and P22 [6]. Surprisingly the carboxy-terminal part of the LexA repressor shares striking homology with one of the SOS genes involved in mutagenesis, the *umuD* gene product [7]. This homology includes the serine and the lysine residues (amino acids 119 and 156 in the case of LexA) thought to constitute the active proteolytic center of LexA [8]. On the contrary, the homology within the cleavage site is not as perfect, in that, the UmuD protein harbors a cysteine-glycine motif instead of the common alanine-glycine dipeptide. We wondered if in the case of LexA a substitution of Ala-84 for a cysteine side chain would be compatible with the cleavage of the repressor. Here we show that the LexA-Cys-84 mutant remains in fact inducible in vivo following UV irradiation and mitomycin C treatment. This indicates that a cysteine-glycine motif is in fact compatible with the cleavage reaction.

Correspondence address: M. Granger-Schnarr, Institut de Biologie Moléculaire et Cellulaire, CNRS and Université Louis Pasteur, 15, rue Descartes, 67084 Strasbourg Cédex, France

2. MATERIALS AND METHODS

Site-directed mutagenesis of the *lexA* gene was performed

essentially as described [9] using an Amersham mutagenesis kit. The single-stranded template used for primer extension was prepared from M13mp19 carrying the *EcoRI*-*Clal* fragment encoding the *lexA* gene from pJL45 [10] cloned into the large *EcoRI*-*AccI* fragment of the phage DNA. Four purified plaques were screened by DNA sequencing by the method of Sanger et al. [11]. All four of them harbored the desired mutation. This high efficiency of the method is due to the elimination of the parental wild-type strand prior to transformation. The *EcoRI*-*PstI* fragment from one of these phages encoding LexA was further cloned into the large *EcoRI*-*PstI* fragment of pBR322, giving plasmid pBLC7, harboring thus the desired substitution of Ala-84 for cysteine. For comparison the wild-type gene has been subcloned in the same manner giving plasmid pBLS1.

The induction of the SOS system *in vivo* was monitored using the *E. coli* strain JL806 (*recA*⁺ *lexA*71::Tn5 Δ (*lacI*POZYA)169 *sulA*11 λ recAop-*lacZ* cl *ind*⁻/F'::Tn3 *lacI*^q *lacZ* Δ M15) [12]. The β -galactosidase activity *A* was measured as described [13] and expressed according to $A = 1000 \times (A_{420} - 1.7 \times A_{550}) / (V \times t \times A_{600})$ [13].

Mitomycin C (from Sigma) induction was performed upon 10-fold dilution of an exponentially growing culture into fresh LB medium containing 2 μ g/ml mitomycin C. UV irradiation was done in MgSO₄ medium using a germicidal lamp (15 W, Philips).

3. RESULTS

In the indicator strain JL806 [12] used in this study, the level of β -galactosidase is controlled by the LexA repressor since its *lacZ* gene has been fused with the *recA* promoter harboring a fairly strong LexA binding site. Furthermore, the strain is devoid of LexA function due to the presence of a transposon within the *lexA* gene. Therefore the level of β -galactosidase will be repressed only if the bacteria are transformed with a plasmid bearing the *lexA* gene. On the other hand, any inducing treatment of the SOS functions will release the β -galactosidase repression as far as the repressor is (self-)cleavable in a RecA dependent way.

Two plasmids, pBLS1 and pBLC7, were introduced separately in this indicator strain. Plasmid pBLS1 bears a wild-type *lexA* gene, while plasmid pBLC7 bears the site-directed LexA mutant in which Ala-84 has been replaced by a cysteine residue (LexA-Cys-84). The construction of both plasmids is described in section 2.

Fig.1 shows the level of β -galactosidase activity when the bacteria transformed with either plasmid are grown in the presence or in the absence of mitomycin C. In both cases we observe a substantial increase in β -galactosidase activity with time after mitomycin C treatment, whereas in the

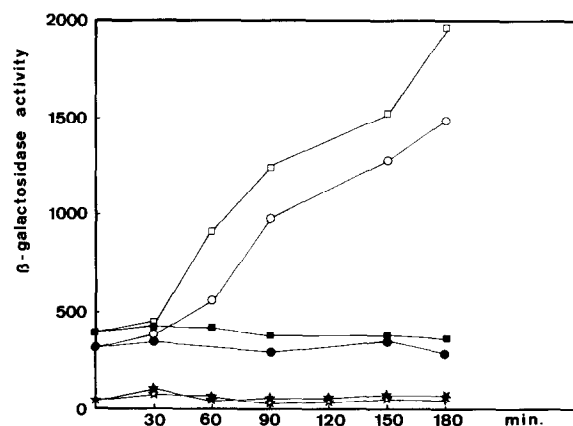


Fig.1. Mitomycin C induced β -galactosidase activity of strain JL806 transformed with plasmid pBLC7 encoding the LexA-Cys-84 mutant (○, ●) or pBLS1 encoding the wild type LexA repressor (□, ■). Open symbols correspond to cultures grown in the presence of 2 μ g/ml mitomycin C, filled symbols to cultures grown in the absence of mitomycin C. JL806 transformed with plasmid pJL118 (☆, ★) encoding the LexA3 mutant is included as an uninducible control.

absence of mitomycin C the β -galactosidase activity remains stably low. This result indicates that, as the wild-type repressor, the LexA-Cys-84 protein may be inactivated upon induction of the SOS system of the host bacteria by mitomycin C treatment. As a control, we used the plasmid pJL118 [12] which bears the *lexA*3 mutant of the LexA repressor. This LexA mutant is essentially un-cleavable (*ind*⁻) due to a mutation of Gly-85 into aspartic acid. As expected, in this case no change in the level of β -galactosidase activity is observed whether the transformed bacteria are grown in the presence or absence of mitomycin C (see fig.1).

As an additional proof for the inactivation of LexA-Cys-84 upon SOS induction, we have used a second inducing treatment, that is irradiation by UV light. Fig.2 shows the results obtained if the transformed bacteria are submitted to a UV dose of 20 J/m². As in the case of mitomycin C induction we observe a substantial increase in β -galactosidase activity for both LexA-Cys-84 and LexA wild-type upon UV irradiation.

Beside the general similarity between LexA-Cys-84 and LexA wild-type, we find that the β -galactosidase activity without any SOS inducing treatment is slightly lower for the LexA-Cys-84 mutant and clearly lower for the LexA3 mutant (figs 1 and 2). The increased repression by LexA3

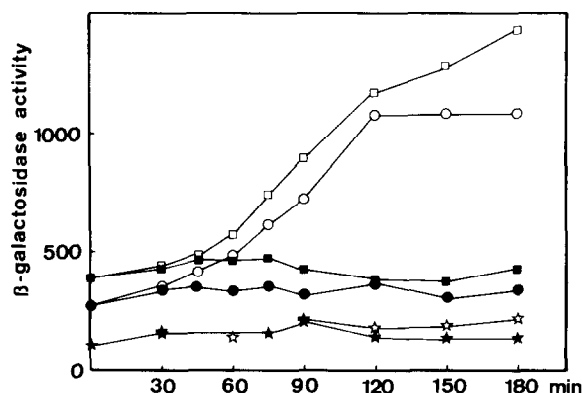


Fig.2. UV irradiation (20 J/m^2) induced β -galactosidase activity of strain JL806 transformed with pBLC7 (○,●), pBLS1 (□,■) and pJL118 (☆,★). Open and filled symbols correspond to irradiated and unirradiated cultures, respectively.

has been observed earlier by Little and Hill [12]. Furthermore, the degree of induction both by mitomycin C and UV irradiation is also slightly lower for the LexA-Cys-84 mutant than for LexA wild-type (figs 1 and 2) suggesting that the cleavage of the Cys-Gly bond is somewhat less efficient than that of the Ala-Gly bond present in the wild-type repressor.

4. DISCUSSION

Using site-directed mutagenesis we have changed the amino acid Ala-84 of the LexA repressor for a cysteine. The reason for this replacement was the striking homology between LexA and UmuD and the fact that alanine and cysteine have side chains of comparable size. Using an *in vivo* repression/induction assay we have shown that: (i) the mutant repressor is not affected in its DNA binding capacity since the degree of repression in the absence of any inducing treatment is even slightly higher than that of the wild-type repressor; (ii) the mutant repressor remains inducible both by mitomycin C and UV irradiation, suggesting that the Cys-Gly bond is nearly as efficiently cleaved as the Ala-Gly bond in the wild-type repressor.

To our knowledge this is the first substitution within the cleavage site of LexA or one of the other inducible repressors that conserves its ability to be induced. Cleavage site mutants being not further inducible have been described for LexA (Asp for Gly) and phage λ repressor (Thr for Ala, Glu and

Arg for Gly) [14]. The only obvious 'rule' that might be drawn from these *ind⁻* mutants is that all the substitutions for Gly are either negatively or positively charged side chains. Clearly the construction of further mutants will be necessary to find out which amino acid side chains are compatible with the cleavage reaction and to define the relationship between the cleavage site and the 'catalytic center' of LexA. The self-cleavage reaction of LexA at alkaline pH is an intramolecular reaction that necessitates a close proximity in space of the cleavage site (Ala-Gly) and the 'catalytic center' of the protein around the active side chains Ser-119 and Lys-156 [8]. Bulky or charged side chains within the cleavage site might in fact hinder the correct positioning of the catalytic center with respect to the cleavage site.

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