In vitro study of the interaction of the LexA repressor and the UvrC protein with a *uvrC* regulatory region

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The in vitro interaction of the LexA repressor with a regulatory region of the *uvrC* gene has been studied by polyacrylamide gel electrophoresis. Although the *uvrC* promoter region shows some homology with the canonic LexA binding site, no specific binding of the repressor to this DNA sequence could be observed, but only a cooperative nonspecific binding. By the same technique we show that the UvrC protein does not bind specifically to this regulatory DNA sequence either, although the protein is able to bind nonspecifically and cooperatively to the double-stranded DNA fragment.

LexA repressor uvrC *gene* *UvrC protein* *Gene expression regulation*

1. INTRODUCTION

In *Escherichia coli*, the removal of a variety of noncoding DNA lesions is performed by the so-called excision repair mechanism. The early step of this mechanism, i.e. the incision step, is dependent on the products of three genes, *uvrA*, *uvrB* and *uvrC* (review [1]). These three proteins act as a complex, incising duplex damaged DNA, which results in two endonucleolytic breaks on either side of the lesion [2,3]. This step is coupled to the removal and resynthesis of DNA in which at least the gene products of *polA*, *uvrD* and *ligA* are involved [4–6]. The concerted action of UvrA, UvrB and UvrC suggests that the genes may be coordinately expressed although the *uvr* genes are not linked. The UV inducible expression of *uvrA* and *uvrB* has been demonstrated and is mediated by the cellular SOS system controlled by a common repressor, LexA [7,8]. Furthermore, the *uvrA* and one of the *uvrB* regulatory regions have been shown to contain typical LexA binding sites [9,10].

However, the case of *uvrC* is still uncertain. The use of multicopy plasmids harboring fusions between regulatory sequences upstream from *uvrC* to *galK* suggests that one of the putative *uvrC* promoters is inducible in a RecA-LexA dependent way [11]. This apparently LexA-controlled promoter contains a sequence located between the '−35' and '−10' sequences of this promoter displaying partial homology with the consensus sequence of a LexA binding site. The *uvrC* sequence $CTGA(N)_{10}CAG$ differs from the consensus LexA binding site $CTGT(N)_9CAG$ by at least two features; (i) the replacement of one of the highly conserved nucleotides (A instead of T) and (ii) a distance of 10 instead of 9 nucleotides between the two highly conserved sequences.

We were therefore interested to determine whether such a sequence could be specifically recognized in vitro by the purified LexA repressor protein.

2. MATERIALS AND METHODS

2.1. *Plasmids*

pJL45 for the purification of LexA was from Little et al. [12], pCA9505 for the preparation of

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the labelled DNA fragment containing the putative LexA box of the *uvrC* gene was from Van Sluis et al. [11] and pJL5 was from Little et al. [12].

2.2. *Purification of LexA and UvrC proteins*

LexA was purified according to Schnarr et al. [13]. UvrC protein was a gift from Dr C. Backendorf (University of Leiden) and was purified according to Zwetsloot et al. [25].

2.3. *Purification and end-labelling of the DNA fragments*

Plasmid DNA was purified according to Clewell and Helinski [14] and Katz et al. [15]. The pCA9505 plasmid was digested with *Bgl*II and *Pvu*II restriction enzymes and the two fragments thus obtained were ^{32}P end-labelled with polynucleotide kinase. The 287 base pair fragment containing the putative LexA binding site was purified by electrophoresis on an 8% polyacrylamide gel under non-denaturing conditions. The DNA fragment was recovered with a low ionic strength buffer. The same procedure was used to obtain the 175 base pair *Hin*dIII-*Eco*RI restriction enzyme fragment of pJL5 plasmid containing the LexA binding site of the *recA* gene.

2.4. *Study of specific interaction by electrophoresis*

The interaction of LexA or UvrC with the respective DNA fragments was studied by polyacrylamide gel electrophoresis as described by Garner and Revzin [16] and Fried and Crothers [17].

3. RESULTS

Fig.1 shows autoradiograms of polyacrylamide gels loaded with ^{32}P radioactive DNA fragments to which various amounts of LexA were added. As previously shown [13] with the 175 base pair long DNA fragment harboring a canonical LexA binding site (fig.1A, lanes a′–d′) a well defined complex with LexA is formed even at protein concentrations as low as 10^{-8} M. Under identical conditions (fig.1A, lanes a–d), no such complex was observed with the 287 base pair long DNA fragment containing the putative *uvrC* LexA binding site. At 10^{-6} M LexA concentration, both DNA fragments show an additional faint band (fig.1A, lanes d and d′) which probably represents a cooperative nonspecific binding. As outlined [13], an isolated nonspecific binding should result in a ladder of bands as was observed with the Lac repressor complexes [17]. Changing the pH to 6.5 instead of 7.4 seems to decrease the amount of the specific binding of LexA while it favors the nonspecific type of binding (cf. fig.1A, lanes d and d′ with B, lanes d and d′). Indeed CD measurements with poly[d(A-T)] confirm a pH dependence of the nonspecific binding (M.

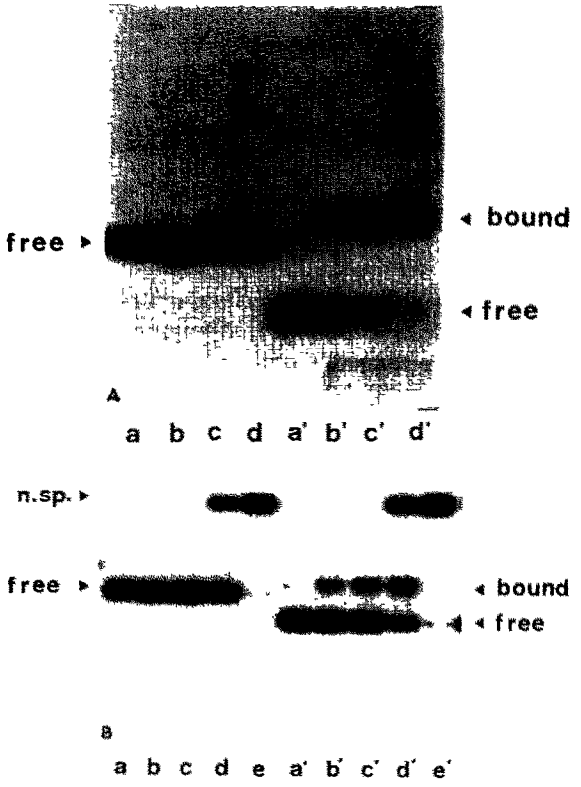

Fig.1. Interaction of LexA with the 287 base pair long *Bgl*II-*Pvu*II restriction enzyme fragment of plasmid pCA9505 (lanes a–e) and with the 175 base pair long *Hin*dIII-*Eco*RI restriction enzyme fragment of plasmid pJL5 harboring the recA operator (lanes a′–e′). LexA monomer concentrations are 0 (a,a′), 10^{-8} (b,b′), 10^{-7} (c,c′), 10^{-6} (d,d′) and 10^{-5} M (e,e′). Panels: (A) the complex is formed in 10 mM Tris-HCl, pH 7.4, 1.6 mM EDTA, 0.04 mg/ml BSA, 20% glycerol and 30 mM NaCl; (B) the complex is formed in 10 mM 1,3-bis(hydroxymethyl)methylaminopropane, pH 6.5, instead of Tris-HCl. n.sp., nonspecific complex. The concentration of the DNA fragment is about 5 nM.

Schnarr, unpublished). The apparent decrease of specific binding at lower pH may be related either to a more severe competition with nonspecific bound species at the operator site, or may reflect a real effect of pH on specific binding opposite to that observed for nonspecific binding.

These results show that the putative LexA binding site of the *uvrC* regulatory region does not specifically bind LexA with an efficiency similar to that of a canonical LexA binding site. The method used does not allow the visualization of binding occurring with a very low binding constant. However, the weakest SOS operator so far described, that of the *lexA* gene, can be easily monitored by this method [13].

The UvrC protein is part of the protein complex involved in the repair of some DNA lesions in conjunction with UvrA and UvrB. The expression of the latter two proteins is under the control of LexA. The possibility remains that the apparent LexA dependence of *uvrC* expression could be an indirect effect. Upon induction, the increased levels of UvrA and UvrB will complex with UvrC and the free intracellular concentration will decrease. Consequently, the *uvrC* gene might have an autoregulatory response to this phenomenon. To address the possibility we executed binding experiments with the purified UvrC protein, the conditions of interaction chosen being either those of LexA with its operator at pH 7.4 or the typical conditions of activity of UvrC (ATP, $MgCl_2$ with or without KCl). Fig.2 shows the results obtained. In panel A, high UvrC concentrations were used. Under various conditions of ionic strength, pH and presence of ATP, we only observed the formation of a nonspecific complex. The absence of a ladder of bands indicates again that this nonspecific binding is cooperative. In panel B of fig.2 we used lower UvrC concentrations and again whatever the conditions used, we could only observe a nonspecific binding of UvrC to this DNA fragment.

These results strongly suggest that the UvrC protein does not bind to this putative regulatory region of the *uvrC* gene in a specific manner. However, they do show that UvrC protein can bind to double-stranded DNA. It can be seen that this nonspecific binding occurs at sufficiently low protein concentrations (10^{-8} to 10^{-7} M) to be potentially important in vivo. Indeed, according to

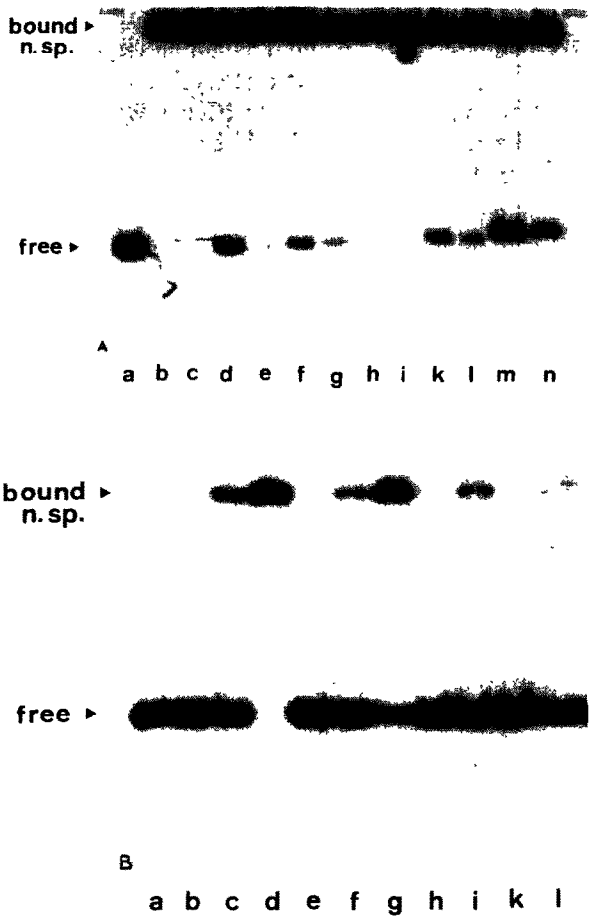

Fig.2. Interaction of the UvrC protein with the 287 base pair long *Bgl*II-*Pvu*II restriction enzyme fragment of plasmid pCA9505. All the samples contain 1 mM DDT and 0.1 mg/ml BSA. The concentration of the DNA fragment is about 5 nM. Panel A: a wide variety of incubation conditions has been used. Lanes: a–g contained 10 mM Tris-HCl, pH 7.4, whereas h–n contained 50 mM; (a) control (no UvrC protein); (c and i) 11.4×10^{-7} M UvrC; (d and k) 5.7×10^{-7} M UvrC, 1 mM ATP, 10 mM $MgCl_2$; (e and l) 11.4×10^{-7} M UvrC, 1 mM ATP, 10 mM $MgCl_2$; (f and m) 5.7×10^{-7} M UvrC, 1 mM ATP, 10 mM $MgCl_2$, 80 mM KCl; (g and n) 11.4×10^{-7} M UvrC, 1 mM ATP, 10 mM $MgCl_2$, 80 mM KCl. Panel B: in these experiments somewhat lower UvrC concentrations have been used. Lanes: a–g contained 10 mM Tris-HCl, pH 7.4, whereas h–l contained 50 mM; (a) control (no UvrC protein); (b and h) 2.9×10^{-9} M UvrC; (c and i) 2.9×10^{-8} M UvrC; (d) 1.4×10^{-7} M UvrC; (e and k) 2.9×10^{-9} M UvrC, 1 mM ATP, 10 mM $MgCl_2$; (f and l) 2.9×10^{-8} M UvrC, 1 mM ATP, 10 mM $MgCl_2$; (g) 1.4×10^{-7} M UvrC, 1 mM ATP, 10 mM $MgCl_2$.

Yoakum and Grossman [19] there are about 10 molecules of UvrC protein in uninduced *uvrC*$^+$ cells. This corresponds to an in vivo concentration of about 10^{-8} M [20]. It has been observed [18] that the UvrC protein is able to bind to single-stranded DNA.

4. DISCUSSION

Upstream of the *uvrC* structural gene, two possible promoter sites have been identified by several laboratories [11,21–23]. A weak promoter is situated close to the start of the structural gene; the second one is located about 280 base pairs ahead of the structural gene. Using *uvrC-galK* fusions, Van Sluis et al. [11] were able to observe an inducible expression of the gene which resembles the SOS response. Upon examination of the nucleotide sequence a possible candidate for a LexA binding site was found overlapping with the distal promoter.

However, we were not able to show a specific binding of LexA on a 287 base pair DNA fragment containing this sequence. Taking into account that the method chosen allowed [13] the detection of a specific binding of LexA with the very weak *lexA* operator, we conclude that this *uvrC* sequence does not function as a specific binding site for LexA. As outlined in section 1, the sequence differs in several aspects from the canonical LexA binding site sequence. Recently, Westman and Mount [24] have shown that a substitution of the T-A base pair in the fourth position of the *recA* operator severely impairs the binding of LexA. The putative binding site of LexA within the *uvrC* regulatory region in fact lacks this apparently necessary base pair. Therefore, if a LexA-controlled regulation of the *uvrC* gene does exist it probably does not occur through binding of LexA within the distal promoter of this gene. It is worthwhile noting that the proximal weak promoter does not show a potential LexA binding site at all [23].

Furthermore, by the same method we were unable to detect a specific binding of UvrC on this DNA fragment. Of course we cannot exclude the possibility that other proteins do bind to regions upstream of the *uvrC* structural gene. If we regard the apparent RecA-LexA dependent regulation of *uvrC-galK* as a valid model for the single copy in vivo situation, regulation might also occur by positive regulation. In this context, we are presently investigating the role of a 24 kDa protein encoded by the DNA sequence situated in the 700 base pair region preceding the *uvrC* structural gene (Van Sluis, in preparation).

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