Cooperative and salt-resistant binding of LexA protein to non-operator DNA

Manfred Schnarr and Michel Daune

Institut de Biologie Moléculaire et Cellulaire du CNRS, Laboratoire de Biophysique, 15 rue René Descartes, 67084 Strasbourg Cédex, France

Received 23 March 1984

The interaction of the *lexA* repressor of *E. coli* with poly[d(A-T)] has been studied by circular dichroism. The binding induces an about 2-fold increase of the circular dichroism intensity at 263 nm, pointing out a conformational change of the nucleic acid. The observed spectral changes are very similar to those observed for the binding of the *lac* repressor to poly[d(A-T)] and natural DNA. At elevated ionic strength the binding isotherms do show a pronounced sigmoidal shape indicating a cooperative mode of binding.

SOS response lexA *repressor* *Protein–nucleic acid interaction* *Cooperativity* *Circular dichroism*

1. INTRODUCTION

The *lexA* repressor (LexA) of *Escherichia coli* plays a central role in the regulation of the so-called 'SOS response' (review [1]). Treatment with radiation or chemical carcinogens of the bacterium leads to cleavage of LexA by the RecA protein (RecA), thereby inactivating LexA and turning on the *SOS* genes. The different operators (SOS boxes) preceding these structural genes share significant homology within at least 7 base pairs out of about 20. At least for the *lexA* gene itself [2,3], and for the colicin E1 gene [4], the operators are composed of two 'SOS boxes' to which the LexA protein binds in a cooperative manner. Here we show that LexA may also bind cooperatively to non-operator DNA. This binding, as revealed by circular dichroism (CD) induces a conformational change of the DNA very similar, if not identical, to that observed for the interaction of *lac* repressor with non-operator DNA. At least at elevated ionic strengths the observed association constants are higher than those reported for any other DNA-binding protein.

2. MATERIALS AND METHODS

The LexA protein has been purified with the aid of the plasmid pJL45 [2] constructed by John Little harbouring the *lexA* gene under the control of the lac UV5 promoter. The detailed purification procedure will be described elsewhere. It consists of Polymin P precipitation of the DNA without co-precipitation of LexA (0.35% Polymin P), ammonium sulfate precipitation of the remaining proteins, phosphocellulose chromatography and a final ammonium sulfate precipitation. The final purity, as judged by SDS-polyacrylamide electrophoresis, was at least 97% (see fig.1). As the LexA protein has no easily measurable activity, the LexA-containing peak in the phosphocellulose elution profile has been identified by electrophoresis.

This preparation was able to bind specifically to operator DNA. Using 5% polyacrylamide gels a ^{32}P-labeled 175-base-pair *Eco*RI/*Hin*dIII fragment (containing the *recA* regulatory region) from the plasmid pJL5 showed decreased mobility in the presence of LexA, whereas the small *Bam*HI/*Sal*I fragment of pBR322 did not.

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00145793/84/$3.00

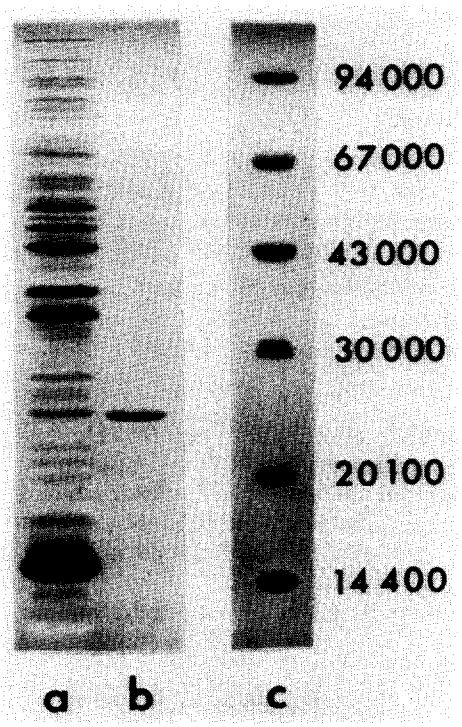

Fig.1. SDS-polyacrylamide (12.5%) gel electrophoresis of the bulk of the proteins before purification (lane a), purified LexA (lane b), and several marker proteins (lane c) with M_r values as indicated.

Using the spectroscopic method in [5] we have determined the molecular extinction coefficient of LexA. We find an absorption ratio of $A_{280\ nm}/A_{205\ nm} = 0.0115$, leading to an extinction coefficient $\epsilon_{280} = 7300\ M^{-1} \cdot cm^{-1}$ per LexA monomer, if we use an M_r of 22300 [6]. This value is rather close to that expected for a protein containing only one tryptophan and one tyrosine residue. The poly[d(A-T)] was from Boehringer (Mannheim) and its concentration was determined using $\epsilon_{260} = 6650\ M^{-1} \cdot cm^{-1}$.

The CD spectra were recorded with a Jobin-Yvon Mark III dichrograph. Each CD spectrum was run at least twice, and we currently checked for eventual baseline shifts.

3. RESULTS AND DISCUSSION

Circular dichroism is a useful tool for the study of protein–DNA interactions if the latter undergoes a conformational change upon binding of the protein. This is apparently the case for the interaction of LexA with poly[d(A-T)]. Fig.2 shows that with increasing amounts of LexA the positive CD band, centered at 263 nm, increases markedly. The contribution of the protein to the total signal in this wavelength range is negligible, whereas it becomes the major one at wavelengths shorter than 240 nm. For this reason only the spectral range between 250 and 310 nm is shown. The observed change of the CD spectrum is very similar to that observed for the interaction of the *lac* repressor of *E. coli* and its NH_2-terminal 'headpiece' with non-operator DNA [7–9], suggesting

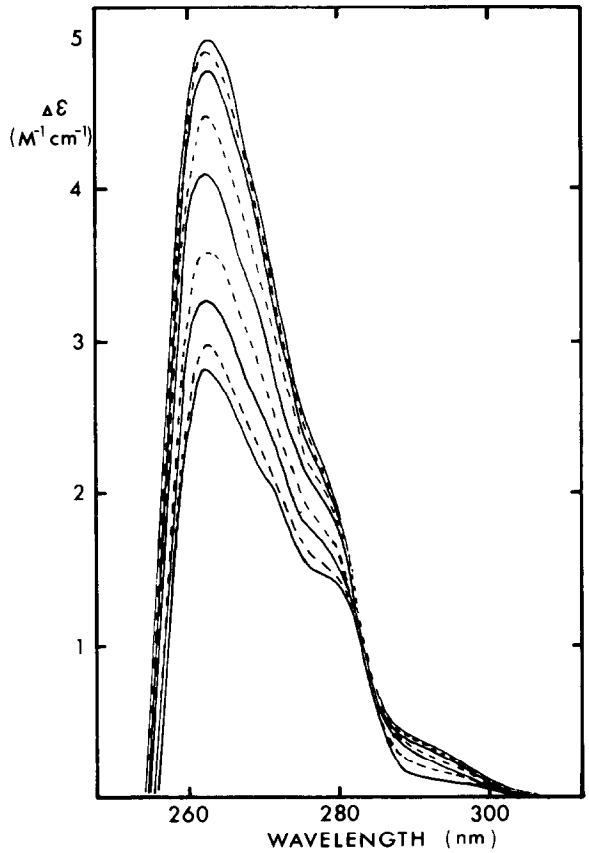

Fig.2. Growth of the CD spectra of poly[d(A-T)] (3.8×10^{-5} M base-pairs; pathlength, 1 cm) upon addition of increasing amounts of LexA protein (0, 1.1, 2.3, 3.3, 4.4, 5.4, 6.5, 7.4, 8.4 μM LexA monomer) at 620 mM NaCl (pH 6.5), 20°C. Spectra are not corrected for the slight dilution resulting from the addition of LexA. Addition of the LexA storage buffer alone leads to no change of the poly[d(A-T)] spectrum.

that both repressors induce the same conformational change of the DNA.

At 400 mM salt the final increase is about 15% smaller than that observed at higher ionic strengths. This may be due to several reasons; for example, part of the LexA molecules may remain in the isolated binding mode and influence the poly[d(A-T)] conformation somewhat differently than do the contiguously bound proteins. Or, if most of the bound proteins remain in the isolated binding mode at this ionic strength, the accumulation of gaps smaller than the binding site size may lead to incomplete saturation of the DNA lattice.

The observed changes of the CD spectra have been used to determine the binding curves at different ionic strengths (fig.3). Above 400 mM NaCl these isotherms adopt a more and more pronounced sigmoidal shape indicating that the binding occurs cooperatively. At lower ionic strength the association constant is apparently high enough to allow for isolated binding. Nevertheless, at very low ionic strength for a given poly[d(A-T)]/LexA ratio we observe a sudden fall of the CD signal (not shown), indicating a reorganisation of the complex, which might be due to a transition from isolated to contiguous binding. Both the binding isotherm at 400 mM NaCl and the central part of the isotherm at 260 mM indicate a binding site size of 8 ± 1 base-pairs per LexA monomer (fig.3). This value is in reasonable agreement with the site size obtained for proteins with a similar number of amino acids like the catabolite gene activator protein (CAP) of *E. coli* (13–15 base-pairs per CAP dimer) [10,11]. Knowing the binding site size and the total protein concentration at half-saturation of the DNA lattice one may calculate the product $K\omega$ [12], K being the association constant and ω the cooperativity parameter. As generally observed for protein–DNA interactions $K\omega$ decreases with increasing ionic strength. Assuming no anion release we determine 7 ± 1 electrostatic interactions upon LexA–poly[d(A-T)] interaction from a log $K\omega$/log[Na^+] plot [13]. We find that the affinity of LexA for non-operator DNA is surprisingly large. Even at 800 mM NaCl, $K\omega$ remains greater than 10^5 M. We are not aware of a comparably strong non-specific protein–DNA interaction under such high-salt conditions. The *lac* repressor of *E. coli* has an affinity of 10^5 M for non-operator DNA at only about 150 mM NaCl [13] and the CAP protein at 200 mM KCl in the presence, and at 100 mM in the absence of cyclic adenosine 3′,5′-monophosphate [11]

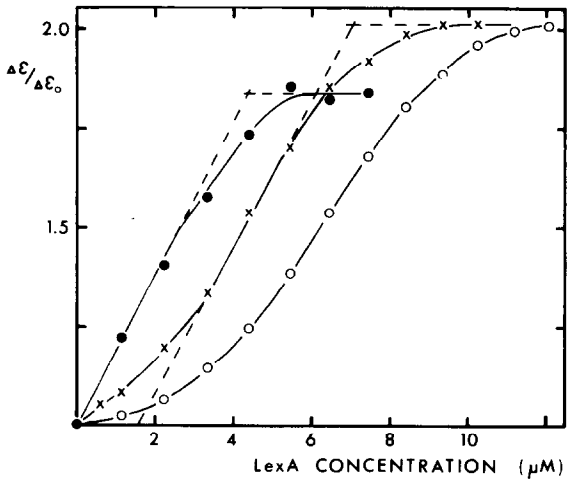

Fig.3. Relative increase $\Delta\epsilon/\Delta\epsilon_0$ of the CD intensity at 263 nm, $\Delta\epsilon$ being the CD intensity in the presence and $\Delta\epsilon_0$ in the absence of LexA, at 3 different NaCl concentrations: 400 mM (●—●); 620 mM (×—×); and 700 mM (○—○).

At present we do not know if the observed strong cooperative interaction of LexA with non-operator DNA has much significance in vivo; for example, whether it mediates a LexA function not detected so far. However, that cooperativity is involved in the specific interaction of LexA with operator DNA has been shown to be the case with the *lexA* and the *col*E1 genes [3,4]. As in the case of the repressor of bacteriophage λ, the cooperative binding to two adjacent sites should enable the system to respond dramatically to a rather modest decrease in repressor concentration [14] and to form a very efficient molecular switch.

ACKNOWLEDGEMENTS

We thank Dr J. Little for the plasmids used in this work and Dr M. Granger-Schnarr for her help in the preparation of the labeled fragments used for the specificity tests.

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