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Structure of UvrABC excinuclease–UV-damaged DNA complexes studied by flow linear dichroism

DNA curved by UvrB and UvrC

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The interaction between UvrABC excinuclease from *Escherichia coli* and ultraviolet light- (UV) damaged DNA was studied by flow linear dichroism. The dichroism signal from DNA was drastically decreased in intensity upon incubation with UvrA and UvrB or whole enzyme in the presence of effector ATP. The change was specific for UV-damaged DNA, and a concluded suppressed DNA orientation suggests the wrapping of DNA around the protein. The incubation with the UvrC subunit alone also somewhat reduces the signal, however, in this case the change was smaller and not specific for UV-damaged DNA. The structural modification of DNA, promoted by the (UvrA–UvrB) complex, probably facilitates or stabilizes the interaction of the UvrC subunit with DNA for the excision.

UvrABC excinuclease; Linear dichroism; Protein–DNA complex; UV damaged DNA; DNA repair

1. INTRODUCTION

In *Escherichia coli*, the UvrABC excinuclease initiates the repair of various adducts of DNA by excising DNA bases on both sides of the adduct [1,2]. The concerted incisions are followed by removal of the damaged oligonucleotide. In presence of ATP, UvrA dimer forms a complex with UvrB ($UvrA_2$-UvrB) that recognizes the damaged site [1]. This complex is further converted into a UvrB–DNA complex, by release of UvrA [3]. Subsequently, UvrC binds to this complex and the damaged strand is incised, respectively, at the 8th and 3rd or 4th phosphodiester bond on the 5′ and 3′ sides of the lesion. The incised oligonucleotide is then released in presence of UvrD and by DNA synthesis the gap is filled.

In order to understand the mechanism, especially the molecular recognition, of this complicated but important process, we have studied the structure of complexes formed between UvrABC and UV damaged DNA using flow linear dichroism (FLD). FLD is an efficient spectroscopic technique for studying interactions and structures of DNA–protein complexes [4–6]. FLD measures the absorption differential of orthogonal forms of linearly polarized light (LD = $A_{\parallel} - A_{\perp}$) on an oriented sample in streaming solution. This measurement provides information about the orientability of a macromolecule and the local orientation of light absorbing chromophores within it (for example, tilt and roll angles of DNA bases). The former property depends on the stiffness and shape of the macromolecule. One can, therefore, detect bent DNA [7,8] and denaturation [9] as well as degradation [10] of DNA by this spectroscopic technique.

Our measurements suggest that both UvrB and UvrC subunits promote curvature of DNA under conditions at which excision has been observed.

2. MATERIALS AND METHODS

The UvrABC excinuclease was reconstituted from individually purified subunits [11]. Usually, the UvrA and B subunits were added, as first step, to DNA in a reaction buffer containing 50 mM Tris-HCl pH 7.5, 100 mM KCl, 10 mM $MgCl_2$, 500 μM ATP, 1 mM dithiothreitol and 100 μg/ml acetylated bovine serum albumin (Biolabs). After 15 min incubation at 25°C, the UvrC subunit was then added and the reaction mixture were further incubated at 25°C for 15 min.

As UV-damaged DNA, 50 mg/ml of T7 coliphage DNA (Sigma, lot 48F-6780) was irradiated in a 1-mm pass length quartz cell with 225 J/m² of 254 nm UV light (this UV dose produces about 6 UV photo products per kilobase pairs). For some experiments, BamHI linearized pSOR plasmid DNA (4360 base pairs length) was used after similar UV irradiation. Qualitatively similar results were obtained.

Flow linear dichroism was measured on a modified Jasco J-500 spectropolarimeter [11] by orientating the sample in an outer rotating

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Abbreviations: UV, ultraviolet light; LD, linear dichroism.

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Couette cell [10,12]. A mild shear of 150 s^{-1} was applied. In this way inactivation of the enzyme was avoided. Also, with smaller shear gradients, far away from saturated orientation, changes in orientability are more sensitively detected [13]. The experiments were carried out at 25°C. Neither albumin nor ATP were found to become orientated under our conditions and, therefore, they did not contribute any linear dichroism signal to be corrected for. We verified that their presence did not distort the linear dichroism spectrum of free DNA above 240 nm although, as a result of high absorption of the solution, large noise prevented evaluation of the LD signal at shorter wavelengths. UvrABC enzyme was gently mixed with DNA in an Eppendorf type of polyethylene test tube in which the solution was incubated at 25°C for 15 min before it was introduced into the Couette cell for measurement. In this way adsorption of free enzyme on the quartz walls of the latter device could be avoided.

3. RESULTS

Fig. 1 shows flow linear dichroism spectra of UV-irradiated and non-irradiated DNA samples (15 μM in base pairs) before and after incubation with UvrA and UvrB subunits in the presence of 500 μM ATP. The LD signal of the UV-damaged DNA is markedly decreased in amplitude, but spectral shape not significantly changed, upon the incubation with 75 nM UvrA and 100 nM UvrB subunits (Fig. 1a). The new spectrum remained stable for at least 10 min after incubation. By contrast, no significant change either in intensity or in shape was observed upon incubation of unmodified DNA with the UvrA and UvrB subunits (Fig. 1b). Since the UvrA subunit can also bind to unmodified DNA [14], this result indicates that the unspecific binding of UvrA does not affect the LD signal of DNA. The addition of a three times larger amount of UvrA alone to unmodified DNA did not significantly change the LD signal. One may further notice that, in the absence of enzyme, there is no large difference between the linear dichroism spectra of UV damaged and undamaged DNA (cf. Fig. 1a and b).

A further decrease of signal amplitude was observed, in the case of damaged DNA, upon additional incubation with 100 nM UvrC subunit, and the signal then practically disappeared (Fig. 1a). Although the last-mentioned effect was observed not to be perfectly reversible, the main spectral changes were cancelled, and the original spectrum essentially recovered, upon the dissociation of proteins by the addition of SDS or by the incubation with proteinase K (not shown). The signal after elimination of proteins was only 15% smaller than that of free DNA. This shows that the main change of dichroism of DNA is indeed due to complex formation with the proteins. The 15% difference indicates the occurrence of some degree of irreversible modification of DNA, most certainly incision, caused by UvrC.

A decrease of signal intensity was observed also with unmodified DNA upon the successive incubations with UvrA, UvrB and UvrC subunits (Fig. 1b). However, the change was smaller than that observed with damaged DNA. Moreover, it was of an extent similar to the

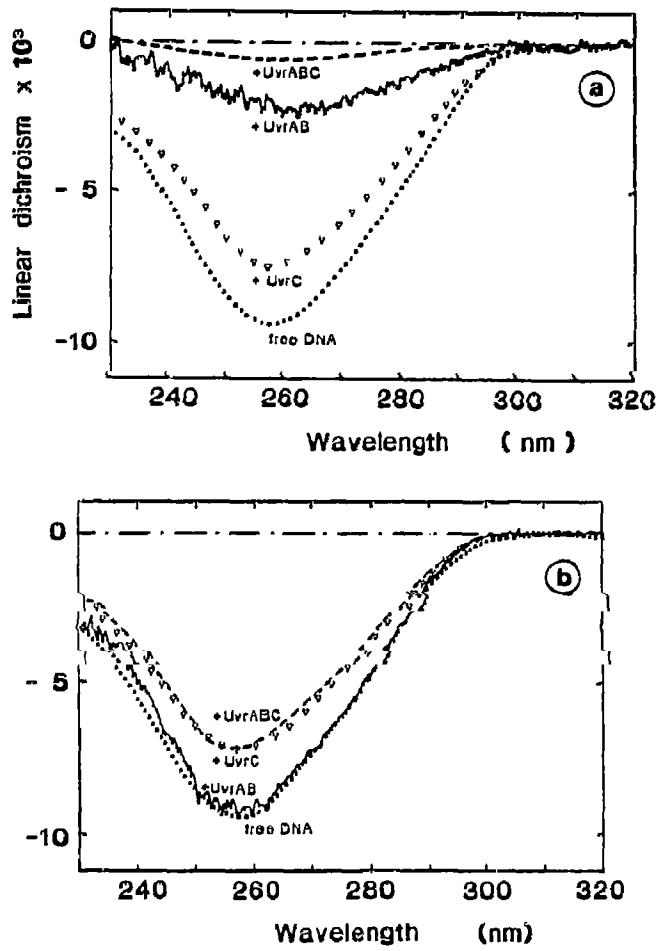

Fig. 1. Linear dichroism spectra of DNA–UvrABC excinuclease complexes; 15 μM (in base pairs) of UV irradiated (panel a) and non-irradiated (panel b) T7 phage DNA incubated with 75 nM UvrA and 100 nM UvrB subunits (———) or, successively, with UvrA and B, and 100 nM UvrC (- - -) or with UvrC subunit alone (▽) as described in text. Linear dichroism was measured at a shear gradient of 150 s^{-1}. The spectrum of free DNA (···) is also shown.

change observed when DNA was incubated with only UvrC subunit (Fig. 1b). The signal change was perfectly reversed upon dissociation of protein. These results indicate that the signal change of unmodified DNA upon interaction with UvrC subunit is due to unspecific protein–DNA binding.

The incubation of DNA with UvrC subunit alone diminished the LD signal intensity, but the degree of change was independent of UV irradiation (Fig. 2). This clearly contrasts the change promoted by UvrAB subunits or the whole enzyme. The latter depends upon the dose of UV irradiation of DNA (Fig. 2) showing that the change in this case is related to a specific interaction of the enzyme with the modified DNA. The change with the UvrC subunit depends only upon the amount of added protein (not shown) and is completely reversed upon the dissociation of protein, confirming the requirement of $UvrA_2$-UvrB complex for the specific in-

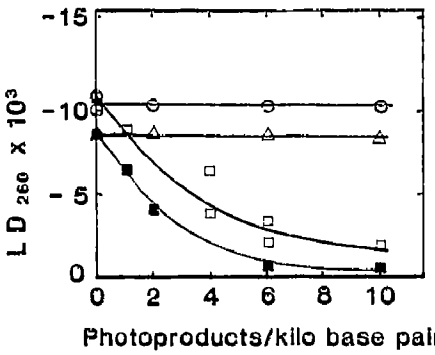

Fig. 2. Effect of UV dose on the change in linear dichroism amplitude of the irradiated DNA by the UvrABC protein system (75 mM UvrA, 100 mM UvrB and 100 mM UvrC). The linear dichroism signals at 260 nm, before (○) and after the incubation with the UvrABC subunits (■), with UvrAB subunits (□), and with UvrC subunit alone (△), are presented as functions of UV dose.

teraction of UvrC subunit and for incision of DNA adduct.

4. DISCUSSION

We here report a decrease in linear dichroism intensity of DNA upon the incubation with UvrA and UvrB subunits and UvrC subunit. With the former subunits the change was specific for UV-damaged DNA, and larger when DNA was irradiated with a larger dose of UV light, while with the latter subunit the change was unspecific and independent of UV dose. The decrease in intensity of linear dichroism could have several origins. One possibility is an altered local DNA base orientation, another is a changed orientability of DNA itself [4,6]. Since the protein/DNA ratio was small (1 UvrA/200 base pairs and 1 UvrB/150 base pairs of DNA) and no change in spectral shape was observed, the large change observed cannot be explained by a modification of local base orientation. Hence, the change must be related to a decrease in the orientability of DNA.

A decrease of orientability, in turn, can be due to the formation of aggregates or compact particles or to the introduction of flexible parts, or to bending (or kinking) of the DNA. The formation of aggregates appears most unprobable because of the very low degree of occupation of DNA by protein. The large change rather suggests a wrapping of DNA around the protein, making the hydrodynamic length of the complex smaller, as was observed in the case of the binding of gyrase [8] and CRP protein [7] to DNA. Both of the latter proteins drastically decreased the steady state intensity of the linear dichroism signal.

Since the UvrA subunit dissociates from DNA after the specific interaction with the DNA adduct has occurred [3], and the unspecific binding of the UvrA subunit does not affect the linear dichroism signal, the signal change observed upon the incubation with UvrA and UvrB subunits must relate to the formation of the UvrB–DNA complex. This conclusion is supported by very recent studies by electron microscopy which indicate a kink of DNA in the complex with UvrB ([15] cited in [16]).

The binding of the UvrC subunit also diminishes the dichroism signal intensity, though to lesser extent than with $UvrA_2$-UvrB complex, suggesting the promotion of a similar structural change, such as bending of DNA upon binding around the protein. Therefore, one may speculate that the particular structural change of DNA, promoted by UvrB, may be the factor that facilitates the recognition of the UvrB–DNA complex by the UvrC subunit or the formation of a stable UvrC–DNA complex.

The slight and irreversible change observed with UV-irradiated DNA upon incubation with the UvrABC subunits is most probably a result of incisions of DNA around thymine dimers, where the two nicks would decrease the stiffness of DNA and thus the intensity of the linear dichroism signal.

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