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Linear Dichroism Study of RecA-DNA Complexes

STRUCTURAL EVIDENCE AND BINDING STOICHIOMETRIES*

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In the presence of RecA single-stranded DNA (ssDNA) is found to exhibit flow linear dichroism (LD). In the absence of the cofactor ATPγS, the LD is positive with a maximum at about 280 nm, whereas in the presence of the cofactor ATPγS there is still a positive long-wavelength band, but a negative LD contribution centered at 260 nm indicates an orientation of the DNA bases preferentially perpendicular to the fiber axis. For the complex between ssDNA and RecA without ATPγS, essentially all LD derives from the protein (tryptophane) subunits indicating a structure in which the tryptophanes are preferentially parallel to the fiber axis of the complex while the DNA bases remain essentially unoriented. The magnitude of the LD increases with the RecA/DNA ratio to a point corresponding to approximately three nucleotides per RecA and decreases thereafter with excess of DNA. This indicates that there are two modes of binding with different stoichiometries.

The RecA protein plays a central role in the DNA repair system of *Escherichia coli*. It is known to promote DNA recombination (1, 2), and to induce the synthesis of a series of proteins required for the DNA repair system "SOS system" by activating the proteolytic cleavage of the LexA repressor (3, 4). RecA isolated in pure form has been shown to exhibit these activities *in vitro* in the presence of Mg^{2+} and ATP (or its nonhydrolyzable analogue adenosine-5′-*O*-(3-thiotriphosphate), ATPγS)[1] as cofactors (1–4).

RecA forms, as judged from electron microscopy, apparently well-structured complexes with single- as well as double-stranded DNA (5–7), probably by entwisting the DNA strands (5). The binding is highly cooperative with a stoichiometry of one RecA per three to four nucleotides (or pairs of nucleotides, in the case of double-stranded DNA) as determined from these observations. In the presence of the cofactor ATPγS, an elongation and a concomitant unwinding of DNA, in contrast to a slight condensation of the complex in the absence of the cofactor, has been reported (5, 6).

In solution an increased fluorescence intensity of a chemically modified DNA (εDNA) has been observed upon binding of RecA, which is further enhanced by the addition of ATP or ATPγS (8, 9). These observations could support the idea of an ATP-dependent conformational change of DNA, induced by the binding of RecA. There may be some controversy regarding the binding stoichiometry of the RecA-DNA complex: experiments in solution have yielded a stoichiometry twice that reported from electron microscopy observations (8–11). A more precise determination of the stoichiometry and structure of the complex is desirable for the understanding of the molecular mechanism of DNA recombination.

In this study we have used flow linear dichroism to approach the structure and stoichiometry of the RecA-DNA complex. This technique has earlier proved successful in providing rather detailed information about the conformation of DNA complexed with small ligands (12–14) as well as about the structure of chromatin *in vitro* (15).

MATERIALS AND METHODS

RecA protein was purified as described elsewhere (16), and its concentration was determined spectrophotometrically using $E^{1\%}_{280} = 5.7$ (17), with a molecular mass of 38,000 daltons (18, 19).

The single-stranded DNA was prepared by rapid cooling of heat-denaturated double-stranded calf thymus DNA (Worthington). All experiments were carried out at 20 °C in a buffer containing 20 mM Tris, 50 mM NaCl, 0.2 mM EDTA, and adjusted to pH 7.6 with HCl. Samples were mixed by adding protein solution to DNA or *vice versa*, and no significant difference was observed due to the order of mixing. When ATPγS (0.5 mM) was present it was added to a DNA-protein solution containing 1 mM $MgCl_2$. All samples were left to stand for 30 min prior to measurements and in no case was any time dependence observed in the monitored LD signals.

The linear dichroism (LD, defined as $A_\parallel - A_\perp$), was measured on a Jasco J-500 spectropolarimeter as described elsewhere (20). The samples were flow oriented in a Couette cell with an inner rotating cylinder according to Wada and Kozawa (21). The LD signal was in all cases found to increase continuously with the flow gradient without any modifications of the spectrum and no artifacts due to turbulent flow could be observed. A constant spin rate corresponding to a shear gradient of 1800 s^{-1} was used in all experiments.

The LD amplitudes, which will here be used only for qualitative considerations, reflect the average orientation of the corresponding light-absorbing transition moment relative to the orientation direction of the complex. LD is positive for a chromophore oriented with its light-absorbing transition moment parallel to the orientation direction (parallel to the flow lines) and negative for perpendicular orientation. Expressed in terms of an "effective angle" $"\theta"$ (defined as $"\theta" = \arccos(\langle\cos^2\theta\rangle)^{1/2}$) between the transition moment and the fiber orientation direction, LD is positive for $0° < "\theta" < 55°$ and negative for $55° < "\theta" < 90°$.

RESULTS

Fig. 1 shows the LD spectrum of the RecA-ssDNA complex (absence of ATPγS). A positive LD band with a maximum at about 280 nm is observed with a shape closely similar to the absorption profile of the pure RecA protein. (For comparison

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[1] The abbreviations used are: ATPγS, adenosine-5′-*O*-(3-thiotriphosphate; ssDNA, single-stranded DNA; LD, linear dichroism.

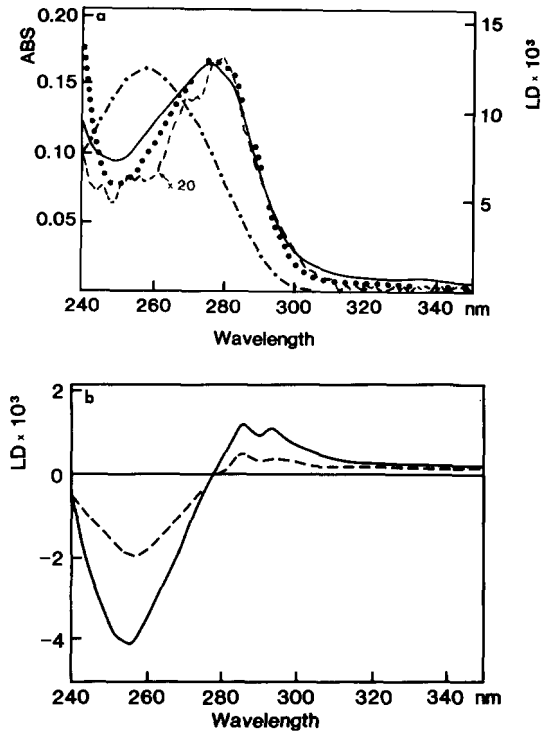

FIG. 1. **Linear dichroism and absorption spectra of RecA-DNA complexes.** a, LD spectra of pure RecA (– – –) and ssDNA-RecA complex (——) and absorption spectra of pure RecA (· · · ·) and ssDNA (–·–·–). Concentrations of RecA and ssDNA are 7 and 21 μM (in Tris-EDTA buffer), respectively. *b*, LD spectra of ssDNA-RecA complexes in the presence of ATPγS (0.5 mM) at nucleotide/RecA ratios of 3:1 (——) and 6:1 (– – –). RecA concentration is 3 μM. The flow gradient is 1800 s^{-1}.

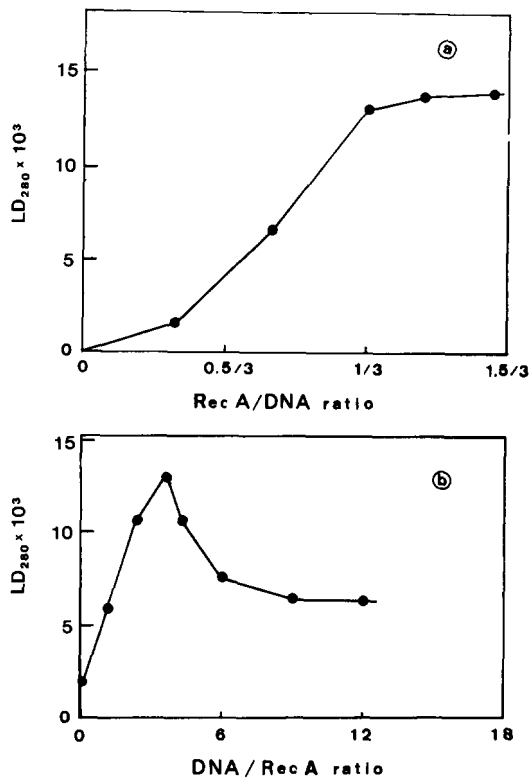

FIG. 2. **Linear dichroism as a function of DNA/RecA ratio.** LD at 280 nm measured after addition of (*a*) RecA to ssDNA (21 μM in nucleotides). *b*, single-stranded DNA to RecA (6.7 μM). Both titrations in Tris-EDTA buffer. The flow gradient is 1800 s^{-1}.

the absorption spectrum of ssDNA, with a maximum at 260 nm, is also shown). Note that pure RecA also exhibits an LD, with a shape similar to that of the RecA-ssDNA complex, however, with about 20 times lower a magnitude. It was checked that the LD observed for free RecA was not due to the presence of spurious amounts of contaminant DNA: upon addition of ATPγS, which dissociates RecA oligomers, the LD disappeared completely.

In the presence of ATPγS, a quite different LD spectrum is observed for the RecA-ssDNA complex (Fig. 1*b*). A positive LD band with a pronounced fine structure, probably of the same origin as the 280-nm band above, is observed around 290 nm. However, at shorter wavelength there is a strongly negative LD band with a maximum at about 260 nm. The ratio of the intensities between the negative and positive LD bands depends on the DNA/RecA ratio: $LD_{255}/LD_{295} = -3.7$ and -5.2 at DNA/RecA = 3:1 and 6:1, respectively.

The LD signal at 280 nm of RecA-DNA in the absence of ATPγS was measured, at a fixed shear gradient of 1800 s^{-1}, at different RecA/DNA stoichiometries. In Fig. 2*a* the results at constant DNA concentration are shown and in Fig. 2*b* at constant RecA concentration. Initially, both graphs show an increasing flow LD with increasing amount of complex. In Fig. 2*a* a sigmoidal increase, virtually leading to a saturation level, is observed, whereas in Fig. 2*b* the signal goes through a maximum corresponding to a DNA/RecA ratio approximately equal to 3 nucleotide units/RecA. Further addition of DNA has the effect of suppressing the LD signal toward a saturation level occurring above approximately 6 nucleotides/RecA.

DISCUSSION

Free ssDNA exhibits an extremely small flow linear dichroism signal due to its high flexibility (22). Under our conditions, the signal is indistinguishable from the base line. In the presence of RecA, a significant positive LD band arises centered around 280 nm (Fig. 1). The shape of this LD band is very similar to the absorption and LD profile of free RecA. We will therefore attribute this LD to oriented RecA proteins. The absorption at 280 nm mainly derives from the presence of two tryptophanes in each RecA protein (18). The positive LD would be consistent with an orientation of the chromophoric indole planes of the tryptophanes preferentially parallel to the fiber axis.

In the presence of the cofactor ATPγS, a strong negative LD band centered around 260 nm develops. This band can be due to either oriented DNA bases, or oriented ATPγS, or both. Work with single-stranded homopolymers,[2] suggests that this band is mainly owing to DNA base absorption, although a contribution from ATPγS cannot be excluded. The negative sign of the signal is consistent with an orientation of the base planes preferentially perpendicular to the fiber axis.

The fact that no LD is observed arising from DNA base absorption in the complex without the cofactor is consistent with a random orientation of the DNA bases. Alternatively it could also be due to an orientation of all transition moments at 55° to the fiber axis. However, since the LD is zero over a wide wavelength region corresponding to a large number of transitions, all with different moment directions in the bases (23), this alternative is considered highly improbable. A random base orientation supports a model where RecA initially interacts mainly with the DNA backbone. Addition of the cofactor may induce structural changes in the protein causing an interaction with the DNA bases yielding a structure in which these become oriented perpendicularly to the fiber axis.

[2] M. Takahashi, M. Kubista, and B. Nordén, unpublished results.

The magnitude of the LD is found to depend on the RecA/ssDNA-binding ratio both with (Fig. 1*b*) and without (Fig. 2) ATPγS. The profile of the positive part of the LD spectrum remains essentially constant over a wide range of binding ratios. This indicates that the orientation of the tryptophanes is essentially the same at low and high occupancy. The constant LD profile in Fig. 1*b* also supports the assumption that anisotropic turbidity does not play any major role in the LD titration in Fig. 2. The quite large magnitude of the LD of the complex, compared to the LD of free RecA, implies a relatively high degree of orientation. Hydrodynamic studies of DNA suggests that a minimum length of a rigid segment (corresponding to the "persistence length") of several hundred Ångströms is required for observable flow orientation.[3] If the site distribution of RecA bound to DNA had been random, one would expect very inefficient orientation properties, since free DNA regions would act as flexible joints. It is therefore reasonable that the high degree of orientation results from the binding of RecA into stiff clusters along the DNA, as is also suggested by electron microscopic observations (5, 6, 7, 24).

The sigmoidal increase of the LD signal at 280 nm (Fig. 2*a*) is apparently not only an effect of a cooperative binding, *i.e.* less efficient binding at low RecA/DNA ratios. In fact, experiments at very low salt concentrations, where the association constant should be considerably enhanced (25), give similar response curves (results not shown), indicating an almost quantitative binding. The sigmoidal behavior is more likely due to the gradually improved orientation efficiency as the DNA/RecA segments become longer. Alternatively, there may be different types of complexes at low and high RecA/DNA ratios. When measuring the magnitude of the LD signal at constant RecA concentration as a function of added DNA (Fig. 2*b*), a virtually linear increase of LD up to a ratio of ~3 nucleotides/RecA is observed, followed by a decrease to about half the maximum magnitude at a ratio of ~6 nucleotides/RecA. Further addition of DNA does not appear to change the magnitude. This indicates that RecA forms a complex with DNA with a stoichiometry of 3 nucleotides/RecA.

The decrease in LD at higher ratios could be due to a rearrangement of RecA binding giving segments of free DNA which should decrease the overall orientation. However, a similar behavior is also observed in the presence of the cofactor ATPγS (results not shown). In the case of ATPγS, the binding of RecA is practically irreversible and excludes the possibility of rearrangement. On the basis of the distinct decrease in LD, we propose that a second complex with a stoichiometry of 6 nucleotides/RecA is formed. The latter complex, which is likely to involve two different DNA strands, is less well oriented as judged from the lower LD (Fig. 1*b*).

The present results could provide a physical basis for explaining the inhibition of RecA-dependent cleavage of the LexA and λ-repressor at high DNA/RecA ratio (16, 26, 27). *In vitro*, these proteolytic events are activated by single-stranded DNA, and the proteolytic rates attain their maximum at a ratio of about 4 nucleotides/RecA and then decrease with excess of DNA. It has been suggested that this inhibition is caused by the formation of a different type of RecA-DNA complex at high DNA/RecA ratio (26). However, the possibility that repressors may be protected by the interaction between the repressor and excess of DNA cannot be ruled out.

The observations described above can also be of help for the understanding of the recombination mechanism: it has been proposed that RecA has two DNA-binding sites, enabling the simultaneous interaction with two DNA molecules (28). The observed doubling of the binding stoichiometry at high DNA/RecA ratio (Fig. 2*b*) is in accord with this hypothesis. The fact that evidence for the proposed complex with the higher stoichiometry (6 nucleotides/RecA) has not been observed from electron microscopy studies might be due to instability of this type of complex which tends to dissociate during sample preparation. Alternatively, the two complexes, in view of their similar structures, could be hard to distinguish from each other with electron microscopy.

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[3] M. Eriksson, unpublished results.

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