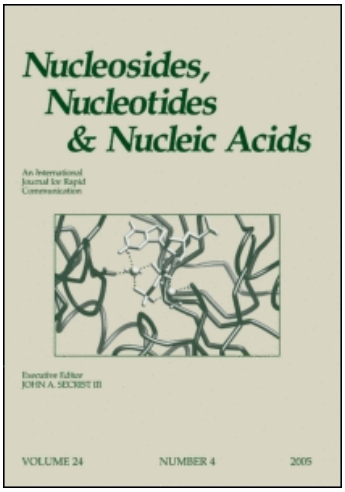

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Competitive Binding Between Unmodified and Etheno DNA Provides Information About Structure and Stoichiometry of RECA-DNA Complexes

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COMPETITIVE BINDING BETWEEN UNMODIFIED AND ETHENO DNA PROVIDES INFORMATION ABOUT STRUCTURE AND STOICHIOMETRY OF RECA-DNA COMPLEXES

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Abstract RecA is found to form three different complexes with single-stranded DNA with stoichiometries of 3, 6, and $\geq$ 9 nucleotides per RecA monomer. In the first two complexes the DNA bases are oriented preferentially perpendicular to the fiber axis of the complex. The second complex is shown to involve two different DNA strands.

RecA protein of *Escherichia coli* is able to mediate important steps in the general genetic recombination.[1] *In vitro*, it forms a complex with single-stranded (ss) DNA with a stoichiometry of ~3.5 nucleotides per recA monomer.[2] From contour length measurements in electron micrographs the length of the complex has been estimated to about 60 % of corresponding double-stranded (ds) DNA.[3] In presence of adenosine 5'-*O*-(3-thiotriphosphate) (ATPγS), a non-hydrolyzable analog of ATP, and excess of RecA, a complex is formed that is extended to about 150 % of corresponding dsDNA. This complex is right-handed helical with a pitch of approximately 95 Å and about six RecA subunits per turn.[4]

We have earlier shown with flow linear dichroism (LD) that the cofactor ATPγS induces structural changes of the RecA-ssDNA complex and that several complexes form in presence of the cofactor.[2,5] In this work we combine LD with fluorescence measurements using ϵDNA (ssDNA with adenine and cytosine residues converted into their etheno derivatives), and poly(dϵA), in order to characterize the number and nature of complexes formed in presence of ATPγS.

Materials and methods

RecA protein was prepared as described elsewhere.[6] ssDNA and ϵDNA was prepared by heat denaturation of dsDNA (Sigma, type I) and chemical modification[7]. All experiments were performed at room temperature in 20 mM Tris-HCl, 2 mM dithiothreitol, 0.2 mM ATPγS (Boehringer Mannheim), 1 mM $MgCl_2$, pH = 7.5.

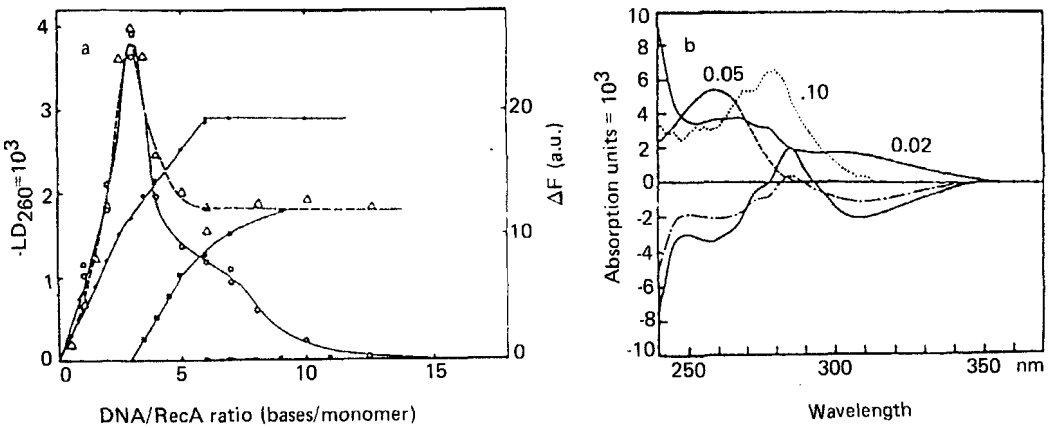

Figure 1. **a**: LD titrations of RecA with ssDNA (o) and ϵDNA (Δ), and fluorescence enhancement of ϵDNA added to 0:1 ($\bullet$), 3:1 ($\cdot$) and 6:1 ($\blacktriangledown$) ssDNA-RecA mixtures. RecA concentration 3μM, flow gradient 1800 s^{-1}. **b**: LD spectrum of 3:1 (———) and 6:1 (—·—) RecA-ϵDNA mixtures, absorption spectra of ATPγS (— —) and poly(dϵA) (———), and LD spectrum of RecA (·····).

Flow linear dichroism was measured on a Jasco J-500 spectropolarimeter and isotropic fluorescence on an Aminco SPF-500 spectrofluorimeter.

Results

FIG. 1a shows the LD signal at 260 nm as a function of ssDNA-RecA and ϵDNA-RecA ratios. For both complexes the LD magnitude increases initially with ratio, reaches a maximal value at about 3 nucleotides per RecA and decreases with further increase in ratio. For the titration with ϵDNA a saturation level is reached at about 6 nucleotides per RecA and with ssDNA an inflection point is observed at this ratio above which the LD magnitude decreases further and eventually vanishes. The fluorescence intensity of ϵDNA increases with a constant slope with the DNA-RecA ratio up to 3 nucleotides per RecA, above which the slope changes to a new value that is maintained up to 6 nucleotides per RecA. Above this ratio no changes in fluorescence intensity are observed. Addition of ϵDNA to a 3:1 ssDNA-RecA mixture decreases the LD magnitude (not shown) and the fluorescence intensity of ϵDNA increases, whereas addition of ϵDNA to a 6:1 ssDNA-RecA mixture does alter neither the dichroism (not shown) nor the fluorescence intensity significantly.

In FIG. 1b LD spectra of 3:1 and 6:1 poly(dϵA)-RecA mixtures are shown. Both spectra display negative LD around 260, a small positive peak at 285, and a negative LD band above 300 nm. The overall shape of the spectra is somewhat different: the ratio $-LD_{310}/LD_{285}$ is about 1 for the 3:1 mixture and about 3 for the 6:1 mixture.

Discussion

The flow LD signal of free ssDNA is extremely small[8] and all observed LD features can be ascribed to complexes with RecA. The LD magnitude is proportional the RecA concentration 3 μM, flow gradient 1800 s^{-1}. amount of complex formed and to the length of the fibers, since longer fibers are expected to orient better in a flow field. The fluorescence intensity enhancement of ϵDNA is expected to be proportional to the amount of complex formed.

The maximal LD magnitude and altered slope of the fluorescence intensity differential at 3 nucleotides per RecA observed for RecA-ϵDNA mixtures (FIG. 1a) indicate that RecA forms a complex with ϵDNA with this stoichiometry. The plateaus in LD and fluorescence intensities at a ratio of 6 nucleotides indicate that a second complex is formed. The constant LD magnitude above this ratio reflects that complexes involving ATPγS are kinetically stable and do not rearrange.[9]

Corresponding complexes appear to form with unmodified ssDNA as judged from the LD maximum at 3 nucleotides per RecA and the inflection point in the LD titration at 6 nucleotides per RecA. The decrease in LD magnitude with further increase in ssDNA/RecA ratio indicates that RecA forms a third complex with unmodified ssDNA that is not formed with ϵDNA. This complex is likely to be unstructured or highly condensed, possibly an aggregate, as judged from the vanishing linear dichroism.

Addition of ϵDNA to a 3:1 ssDNA/RecA mixture increases the fluorescence intensity of ϵDNA whereas addition to a 6:1 ssDNA/RecA mixture does not alter the fluorescence intensity at all. This indicates that ϵDNA is coordinated by the 3:1 but not by the 6:1 ssDNA/RecA complex and we suggest that the 6:1 complex involves two separate strands of ssDNA with an overall stoichiometry of 3:3:1.

The negative LD above 300 nm (FIG. 1b) can safely be ascribed to poly(dϵA) since neither RecA nor ATPγS has significant absorption in this region. The transition moments in the modified bases are expected to be in-plane polarized ($\pi \rightarrow \pi^*$ transitions) and the negative LD is consistent with a base orientation preferentially perpendicular to the fiber axis. The positive LD at 285 nm is likely owing to tryptophane residues in RecA which are known to have positive LD in RecA-ssDNA complexes without ATPγS and in pure RecA fibers.[2] The corresponding transition moments in tryptophane are polarized in the plane of the chromophoric indole part and the positive LD is consistent with an orientation of the indole planes preferentially parallel to the fiber. The LD around 260 nm can have contributions from RecA, poly(dϵA), and ATPγS. Since the LD contributions from the constituents do not necessarily have the same wavelength dependence as

the corresponding absorption spectra, the LD spectrum is hard to resolve. However, the relatively strong LD of poly(dεA) above 300 nm indicates that the contribution from poly(dεA) also dominates the region around 260 nm.

The magnitude of the ratio LD_{310}/LD_{285} is larger for the 3:3:1 than for the 3:1 complex. Although the LD signal at 285 nm is not entirely due to RecA, the increase in ratio is consistent with a larger LD contribution from poly(dεA), relative to the LD from the tryptophane residues, in the 3:3:1 complex. If the tryptophane orientation in these complexes is essentially the same, this indicates that the DNA bases in both strands in the 3:3:1 complex are oriented preferentially perpendicular to the RecA-ssDNA fiber.

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