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Binding of RecA Protein to Z-form DNA Studied with Circular and Linear Dichroism Spectroscopy*

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Binding of RecA to poly(dG-m^{5}dC) and poly(dG-dC) under B- and Z-form conditions was studied using circular dichroism (CD) and linear dichroism (LD). LD revealed a quantitative binding of RecA to Mg^{2+}-induced Z-form poly(dG-m^{5}dC) with a stoichiometry of 3.1 base pairs/RecA monomer, which is slightly larger than the 2.7 base pairs observed for the B-form. The LD spectra indicate a preferentially perpendicular orientation of DNA bases and a rather parallel orientation of the tryptophan residues relative to the fiber axis in both complexes. The association rate of RecA to Z-form DNA was found to be slower than to B-form. CD measurements showed that the polynucleotide conformation is retained upon RecA binding, and CD and LD confirm that RecA binds to both forms of DNA.

The Mg^{2+}-induced Z-form is shown to be retransformed into B-form, both in free and in RecA-complexed polynucleotides by addition of NaCl, whereas the B → Z transition cannot be induced by addition of Mg^{2+} when the polynucleotide is complexed with RecA. From this it is inferred that RecA does not stabilize the Z-conformation of the polynucleotide but that it can kinetically "freeze" the polynucleotide in its B-conformation. On all essential points, the same conclusions were also reached in a corresponding study of unmethylated poly(dG-dC) with the Z-form induced by Mn^{2+}.

In *Escherichia coli*, the product of the RecA gene is required for general genetic recombination (for reviews see Refs. 1–4). In relation to this activity, purified RecA protein in the presence of ATP as a cofactor promotes strand transfer between two homologous DNAs *in vitro* (2–5). This finding has led to the use of the RecA protein as a model in the study of the molecular mechanisms of the recombination reaction. Structural studies of RecA·DNA complexes were pursued by electron microscopy (3, 4, 6, 7), later improved by image analysis (8, 9), and recently complemented by spectroscopic investigations in solution (10–12). Studies of kinetics and binding equilibria of DNA·RecA interactions were also undertaken (2–4, 13–18).

RecA is known to bind to double-stranded DNA in presence of the cofactor ATP (or its analog ATPγS)[1] (19) with extremely slow rates (17), and it interacts with single-stranded DNA both in the presence and absence of the cofactor (3, 4, 13–16, 19). In all complexes, RecA monomers seem to be arranged in a helical manner around the DNA (2–4, 6, 7). The complexes are much stiffer than the corresponding free DNA as judged by image analysis of electron micrographs (8, 9) and flow linear dichroism (LD) measurements (10–12). The LD signals of the complexes are extremely large and therefore suitable for both structural analysis and kinetic studies. The RecA complex with double-stranded DNA in the presence of ATPγS appears to be stretched (2–4, 7) and the DNA bases are well oriented (10–12) and unwound (6, 20), in contrast to the complex with single-stranded DNA in absence of ATPγS in which the DNA is slightly condensed (4, 7) and the DNA bases apparently unoriented (10–12).

It was recently reported that RecA binds with higher affinity to UV-irradiated DNA (21) and Z-form DNA (22). The latter result is particularly interesting in relation to recombinase activity: in the course of strand exchange between two DNAs, it may be speculated that one DNA strand is in a left-handed configuration to form a double-helix with the complementary strand of another DNA molecule (23). Electron microscopy studies give some support to this hypothesis (24).

In this work we study the binding of RecA to Z-DNA using spectroscopic measurements (CD and LD) which provide information about hydrodynamic properties and structure but also about the conformation of the DNA in the complexes. Kinetic studies are also undertaken and the results compared with the binding kinetics with B-form DNA under corresponding conditions.

MATERIALS AND METHODS

RecA was purified as described elsewhere (18, 25) except that a larger phosphocellulose column was used in order to eliminate impurities that may aggregate the RecA and RecA·DNA complexes. Under our experimental conditions, no significant turbidity was observed in CD above 310 nm. However, at NaCl concentrations below 30 mM a significant turbidity was noticed, especially in the presence of $MnCl_2$. Protein concentrations were determined spectroscopically using $\epsilon_{280} = 2.17 \times 10^4$ M^{-1} cm^{-1} (26). Poly(dG-dC) (lot OK 617910) and poly(dG-m^{5}dC) (lot OC 617938) were obtained from Pharmacia LKB Biotechnology Inc. and used without further purification. Their concentrations were determined using $\epsilon_{254} = 8.4 \times 10^3$ M^{-1} cm^{-1} (in bases) and $\epsilon_{259} = 7.0 \times 10^3$ M^{-1} cm^{-1}, respectively.

CD and LD were measured on a Jasco J-500 spectropolarimeter. LD measurements were carried out as described earlier (10–12, 27) using an inner rotor Couette cell (28) at a shear gradient of 600 s^{-1}. CD and LD measurements were conducted in a buffer containing 20 mM Tris-HCl, 40 mM NaCl, and 1 mM dithiothreitol, pH 6.9, at 20 °C, supplemented with indicated concentrations of $MgCl_2$ or $MnCl_2$. The concentration of ATPγS (Boehringer Mannheim) was usually 20 μM. Higher concentration of ATPγS could not be used because of distortion of CD spectra due to large absorption. However, 20 μM of ATPγS is expected to be more than sufficient to saturate 8–10 μM RecA that has been used here, because the dissociation constant of ATPγS·

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[1] The abbreviation used is: ATPγS, adenosine 5′-*O*-(thio)triphosphate.

RecA is about 10^{-7} M (29, 30) and is expected to be even smaller in the presence of DNA. It was further verified by LD that an increase of the ATPγS from 20 to 200 μM did not significantly affect the association kinetics or the LD spectrum.

The DNA-RecA binding reaction was generally initiated by addition of ATPγS to an equilibrated DNA/RecA mixture, although it was found that the order of addition of RecA, DNA, ATPγS, and $MgCl_2$ does not affect the kinetics of RecA binding to double-stranded DNA,[2] which is in contrast to the binding of RecA to single-stranded DNA (16). For LD measurements, the solution was mixed in a test tube and immediately transferred to the Couette cell by aid of a syringe, enabling LD to be monitored 1–2 min after the mixing. In all cases, the mixing time is referred to as time 0, and the binding rates are characterized by half-times ($\tau_{1/2}$) referring to 50% of the total signal change. During the measurements, the shear was occasionally removed to verify base-line stability.

RESULTS

B → Z-transition of Poly(dG-m⁵dC)—Methylated poly(dG-dC), referred to as poly(dG-m⁵dC), undergoes B → Z-transition at much lower Mg^{2+} concentrations (31) than unmodified poly(dG-dC) (32). Under our conditions (40 mM NaCl), poly(dG-m⁵dC) was transformed to Z-form by the presence of about 1.3 mM $MgCl_2$ (Figs. 1 and 2). The rate of the B → Z transition was found to be very slow, especially at low $MgCl_2$ concentrations (Fig. 1). In 0.5 mM $MgCl_2$, no significant amount of DNA in Z-conformation could be detected even after 3 h of incubation (Figs. 1 and 2*b*). Therefore, it was possible to study the binding of RecA to both B- and Z-forms of the same DNA by changing the $MgCl_2$ concentration. The use of the same DNA is crucial for a strict quantitative comparison since the binding of RecA to DNA can be anticipated to depend strongly on the base composition and chemical modification of the bases, as inferred from the case of single-stranded DNA (13, 15). The interaction of RecA with single-stranded DNA appears to be weaker with shorter DNA (3). Also, with double-stranded DNA the interaction appears to depend on the length of the DNA (22) and on chemical modification by UV irradiation (21). In contrast, the $MgCl_2$ concentration appears not to be crucial: changing the concentration from 0.5 to 5 mM does not significantly alter the binding kinetics or LD signal of RecA-double-stranded DNA complexes.[2]

Since the rate of the B → Z-transition of poly(dG-m⁵dC) is very slow in 1.3 mM $MgCl_2$ (Fig. 1), the binding to both B- and Z-forms could be studied at this $MgCl_2$ concentration. Binding to B-form was monitored immediately after the addition of $MgCl_2$ to the polynucleotide, *i.e.* before the transition to Z-form reached a significant level. Under the same conditions, binding to the Z-form could be studied after incubation with $MgCl_2$ for times sufficient to complete the transition to the Z-form. Thus, an unambiguous comparison between the binding to Z- and B-forms of poly(dG-m⁵dC) was possible.

Conservation of Polynucleotide Conformation upon RecA Binding—Poly(dG-m⁵dC) was incubated for at least 4 h in 1.3 or 2.5 mM $MgCl_2$ to ensure complete transformation to Z-form. The CD spectra were essentially the same at these $MgCl_2$ concentrations (Fig. 2*a*) and were only slightly modified by addition of ATPγS·RecA, even after 10 h of incubation (Fig. 2*a*). The relatively modest changes in CD, especially when one takes into account the contribution from RecA·ATPγS (Fig. 2*a*), indicate a completely retained Z nucleotide conformation in the complexes with RecA·ATPγS.

The binding to the B-form of the polynucleotide was studied in 0.5 and 1.3 mM $MgCl_2$ (in 1.3 mM $MgCl_2$ the RecA was added immediately after the $MgCl_2$, before the slow transformation to Z-form had occurred). The CD spectra after 10 h of incubation are shown in Fig. 2*b*. There is no significant

[2] M. Takahashi, M. Kubista, and B. Nordén, manuscript in preparation.

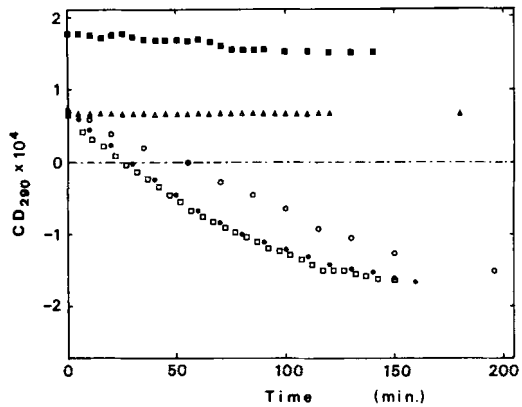


FIG. 1. **Magnesium-induced B → Z transition of poly(dG-m⁵dC).** 25 μM of poly(dG-m⁵dC) in 20 mM Tris-HCl, 40 mM NaCl, pH 6.9, 20 °C incubated with 0.5 mM (Δ), 1.3 mM (○), 1.5 mM (*), 2.5 mM (□) $MgCl_2$, and poly(dG-m⁵dC)·RecA·ATPγS complex incubated with 2.5 mM $MgCl_2$ (■). The transition is monitored by CD at 290 nm.

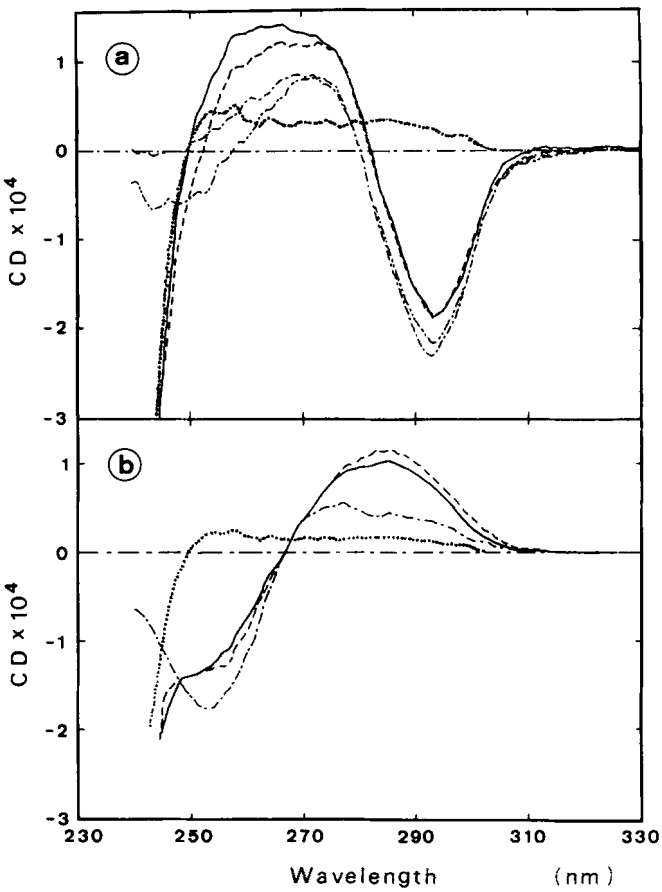


FIG. 2. **CD spectra of RecA•poly(dG-m⁵dC) complexes.** CD (absorbance units) of 25 μM poly(dG-m⁵dC) and 8 μM RecA measured after 10 h incubation in 20 mM Tris-HCl, 40 mM NaCl, pH 6.9, 20 °C at various concentrations of $MgCl_2$. *a*, Z-conformation of free poly(dG-m⁵dC) in 1.3 (– · –) and 2.5 mM (– ·· –) $MgCl_2$ and in complex with RecA in 1.3 mM (– – –) and 2.5 mM $MgCl_2$ (—). The CD spectrum of ATPγS·RecA in absence of DNA is also shown (· · · ·). *b*, B-conformation of free poly(dG-m⁵dC) (– · –) in 0.5 mM $MgCl_2$ and in complex with RecA in 0.5 mM (—) and 1.3 mM (– – –) $MgCl_2$. The spectrum of ATPγS·RecA (· · · ·) is also shown.

difference between the spectra at these two $MgCl_2$ concentrations, but they are clearly different from those of the RecA complex with the polynucleotide in the Z-conformation. During the incubation, the positive band around 280 nm increases and the negative band centered at 250 nm decreases but the overall changes of the CD spectrum are moderate, indicating that the polynucleotide remains in the right-handed B-conformation.

The amount of RecA used in these experiments was 1 monomer/3 base pairs of polynucleotide, which corresponds to the binding stoichiometry for the complex with natural double-stranded DNA (3, 4). A doubling of the RecA concentration modified the CD signals by less than 10% (not shown).

Binding Stoichiometries—The binding of RecA to double-stranded calf thymus DNA increases the amplitude of the LD signal at 260 nm by a factor of 18 and modifies the shape of the LD spectrum (12). Such a large spectral change strongly facilitates accurate quantitative determination of stoichiometry as compared to the use of the small changes in CD spectrum.

Various amounts of polynucleotide were mixed with 0.8 μM RecA, incubated for 10 h at 20 °C, and LD spectra were recorded. For the Z-form of the polynucleotide the presence of RecA strongly enhanced the negative LD around 260 nm, and a small positive signal above 290 nm appeared. Also, with the B-form polynucleotide the amplitude of the LD signal around 260 nm increased drastically upon addition of RecA, but in this case the signal at 290 nm remained negative (Fig. 3).

For both nucleotide conformations the LD signal at 260 nm initially increased linearly with the polynucleotide/RecA ratio (Fig. 4). In both cases, addition of larger amounts of polynucleotide changed the slope to a value corresponding to that of the free polynucleotide. The cross-over of the extrapolated initial and final slopes (Fig. 4) corresponds to a stoichiometry of 1 RecA/2.7 base pairs of polynucleotides in B-form and 3.1 base pairs for the Z-form. These values are not significantly different from the 3 base pairs/RecA observed for natural double-stranded DNA (12). Note that the LD signal at 260 nm of free Z-form polynucleotide is about twice that of the free B-form (Fig. 3), as has been observed previously (33), and that the LD increases upon RecA binding by a factor of 8.5 for the Z-form but about a factor of 40 for the B-form.

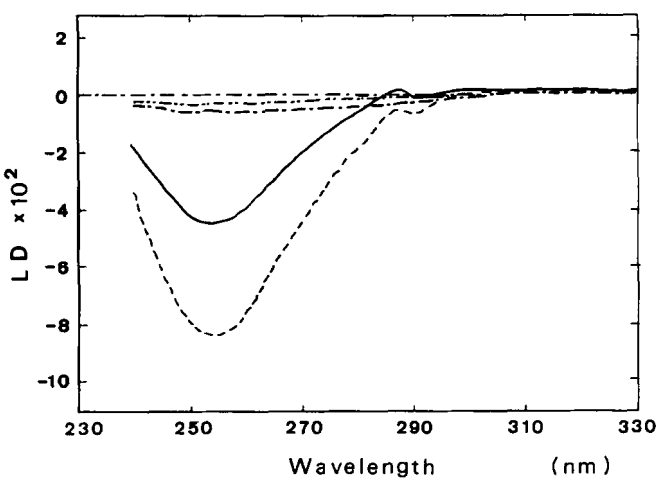


FIG. 3. **LD spectra of RecA•poly(dG-m^{5}dC) complexes.** Free Z- (– · –) and B-form (– · · –) of poly(dG-m^{5}dC) and Z- (—) and B-form (– – –) in complex with RecA. The Z- and B-forms were obtained in 2.5 and 0.5 mM $MgCl_2$, respectively. Shear gradient was 600 s^{-1}. Same conditions as in Fig. 2.

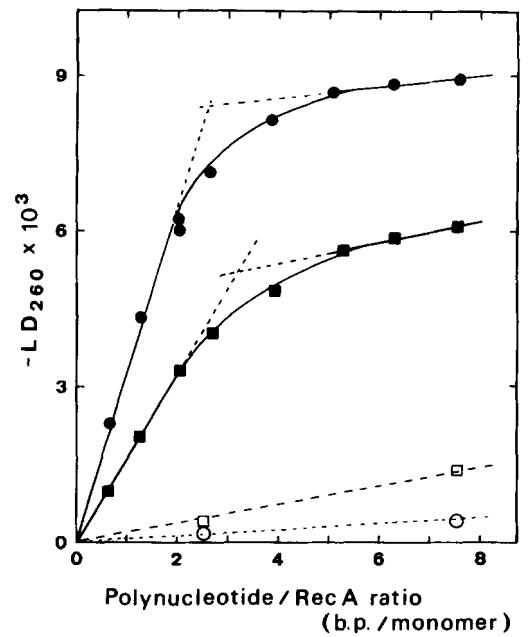


FIG. 4. **Determination of binding stoichiometry of RecA complexes with B- and Z-forms of poly(dG-m^{5}dC).** LD at 260 nm of 0.8 μM RecA mixed with various amounts of B- (●) and Z (■)-forms of poly(dG-m^{5}dC) and LD of free B- (○) and Z (□)-forms of poly(dG-m^{5}dC). Shear gradient was 600 s^{-1}. Same conditions as in Fig. 2. *b.p.*, base pairs.

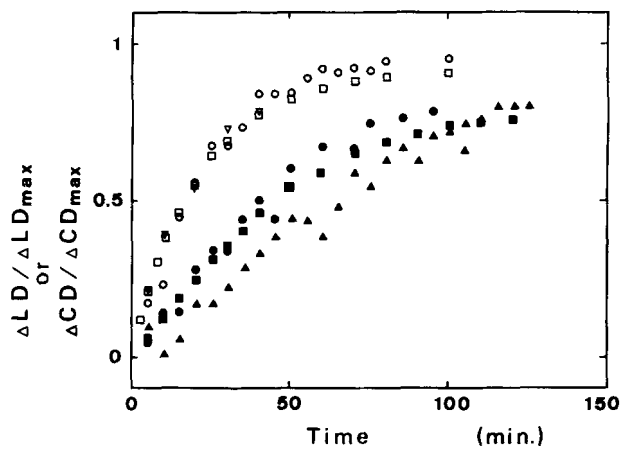


FIG. 5. **Association kinetics of RecA binding to B- and Z-form of poly(dG-m^{5}dC).** The relative changes with time of CD and LD expressed as (signal$_{t=t}$ − signal$_{t=0}$)/(signal$_{t=10\,h}$ − signal$_{t=0}$) of RecA bound to poly(dG-m^{5}dC). Changes in CD (○) and LD (□) after addition of RecA to the B-form in 0.5 mM $MgCl_2$, and changes in CD after addition to the B- (▽) and Z-form (▲) in 1.3 mM $MgCl_2$ and in CD (●) and LD (■) after addition to Z-form in 2.5 mM $MgCl_2$. LD was monitored at 260 nm and CD at 290 and 280 nm for the Z- and B-forms, respectively. Same conditions as in Figs. 2 and 3.

The LD signal of the RecA complex with Z-form is thus about one-half of that with B-form.

Association Kinetics—In order to compare the binding rates of RecA to Z- and B-form of poly(dG-m^{5}dC), the changes in CD and LD signals were normalized, assuming complete binding after 10 h of incubation. The rates determined with LD and CD were in good agreement with each other for both polynucleotide conformations (Fig. 5), although the accuracy is believed to be better in the LD study because of the larger spectral changes. The binding rate was significantly slower to the Z-form of the polynucleotide: the half-time was found to be about 20 min for the binding to the B-form and about 40

min to the Z-form. For neither conformation did the $MgCl_2$ concentration significantly influence the binding rate (Fig. 5).

Differences in Stability between Complexes with Z- and B-form DNA—The Z-form of poly(dG-m^5dC) in 2.5 mM $MgCl_2$ could be transformed back to B-form by addition of NaCl (Fig. 6). In 140 mM NaCl, complete retransformation of uncomplexed poly(dG-m^5dC) into B-form took about 30 min (results not shown). In less than 100 mM NaCl, no significant change in CD spectrum was observed even after 15 min of incubation and only a minor increase in CD was observed in 120 mM NaCl (Fig. 6). The RecA complex with Z-form of poly(dG-m^5dC) in 2.5 mM $MgCl_2$ could also be retransformed to B-form in 140 mM NaCl (Fig. 6) within 30 min, and a small degree of retransformation was observed even in 120 mM NaCl (Fig. 6). Thus, the binding of RecA does not stabilize the Z-conformation of the polynucleotide. The CD spectrum of the fully retransformed polynucleotide in 200 mM NaCl was similar to that of the RecA complex formed directly with B-form of poly(dG-m^5dC), although the intensity around 280 nm was slightly weaker. This suggests that most of the RecA remained bound to the polynucleotide after the transformation. The presence of 200 mM NaCl had no effect *per se* on the structure of the RecA complex with the B-form of poly(dG-m^5dC) as judged from CD (Fig. 6).

Somewhat surprisingly, an increase of $MgCl_2$ concentration to 2.5 mM did not transform the polynucleotide in the RecA complex from B-form into Z-conformation even after 2 h of incubation (Fig. 1). Such an increase in $MgCl_2$ concentration completely transforms the free polynucleotide to Z-form within 2 h (Fig. 1). Even an increase to 10 mM $MgCl_2$ did not cause any significant transformation as judged from CD and LD (not shown). These results indicate that the RecA complex with B-form of poly(dG-m^5dC) is kinetically stable.

RecA Binding to Z-form Poly(dG-dC) Induced by Mn^{2+}—We also carried out experiments with unmodified poly(dG-dC) using Mn^{2+} as a B → Z inducer (34). CD spectra of poly(dG-dC) in the presence of 2.5 mM $MnCl_2$ indicated a B-type conformation of the polynucleotide (Fig. 7*b*), although the positive band around 280 nm was smaller than in the absence of $MnCl_2$ (Fig. 7*c*). Heating the poly(dG-dC) to 60 °C inverted the initially B-form CD spectrum to a Z-form spectrum within 5 min (not shown). After progressive cooling to ensure double-helical structure, the CD around 290 nm remained negative, indicating a retained Z-type conformation (Fig. 7*a*), although the negative band around 290 nm was smaller than for the Z-form induced by $MgCl_2$, as observed previously (34). The polynucleotide remained in the Z-conformation for at least 48 h when kept at 20 °C. Thus, the RecA binding to B- and Z-form poly(dG-dC) could be compared under identical experimental conditions.

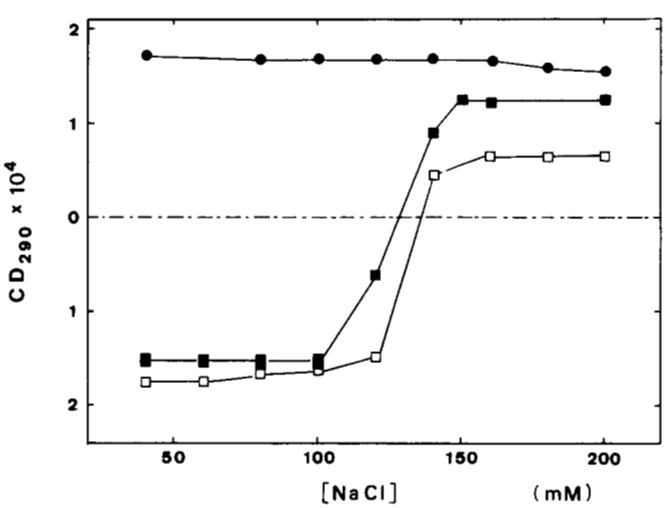


FIG. 6. **Conformation stability of the Z-form of poly(dG-m^5dC) toward addition of NaCl.** CD at 290 nm as a function of NaCl added to RecA·poly(dG-m^5dC) complex (■) and of free Z-form polynucleotide (□) in 2.5 mM $MgCl_2$. For comparison the changes in CD with [NaCl] of RecA complex with B-form poly(dG-m^5dC) in 0.5 mM $MgCl_2$ (●) are shown. Same conditions as in Fig. 2.

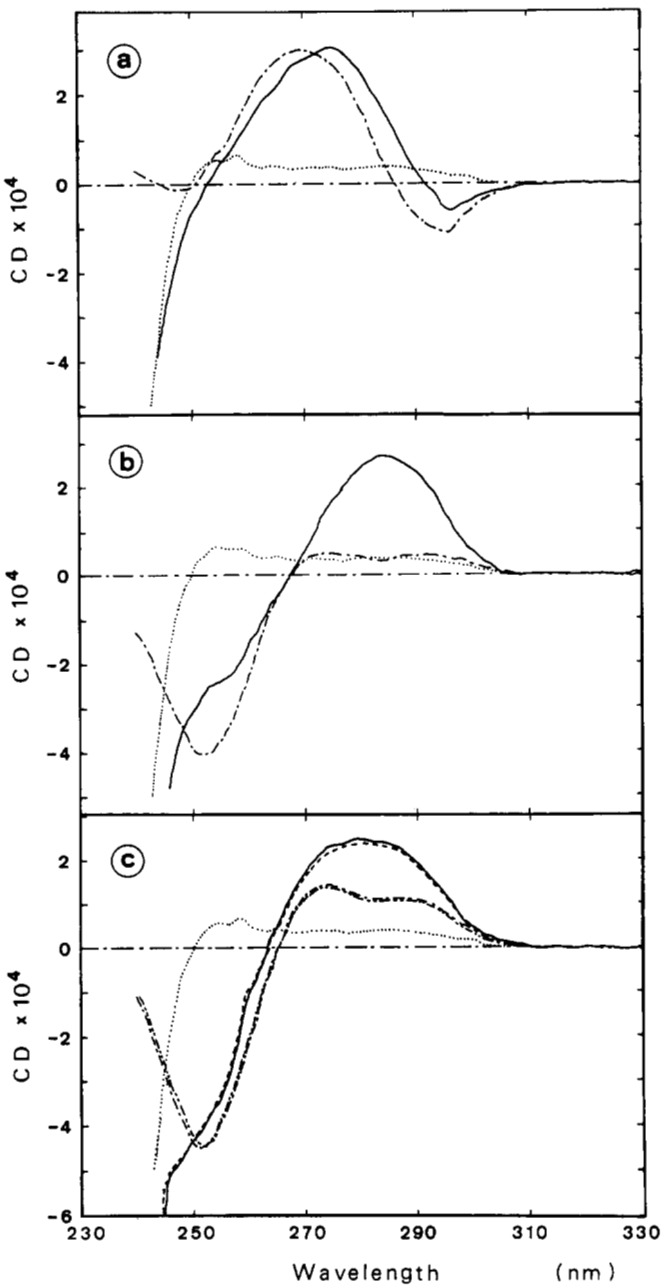


FIG. 7. **CD spectra of RecA•poly(dG-dC) complexes.** *a*, CD of 25 μM free Z-form of poly(dG-dC) induced by heating to 60 °C in 2.5 mM $MnCl_2$ (- · -) and after addition of ATPγS·RecA (—). CD of 10 μM RecA·ATPγS is also shown (····). Same conditions as in Fig. 2. *b*, CD of free B-form poly(dG-dC) (- · -), in complex with RecA (—), and free RecA with ATPγS (····). Same conditions as in *a*, except that the polynucleotide was not heated. *c*, same conditions as in *b* but 2.5 mM $MnCl_2$ was replaced by 2.5 mM $MgCl_2$. CD of free B-form poly(dG-dC) in 2.5 mM $MgCl_2$, with (- · -) and without (- · · -) preheating, and in complex with RecA, with (- - -) and without (—) preheating. CD of free RecA with ATPγS is also shown (····).

Addition of 10 μM RecA and ATPγS to the Z-form of poly(dG-dC) slowly gave a slightly modified spectrum. The change of the CD at 290 nm was larger and occurred somewhat faster than for the RecA complex with the Z-form of poly(dG-m^{5}dC) (Fig. 8). After 10 h of incubation the positive CD band around 270 nm was shifted slightly toward longer wavelengths but the small negative band around 300 nm remained (Fig. 7*a*), indicating that the polynucleotide still was in a Z-like conformation. Addition of RecA to the B-form of the polynucleotide increased the magnitude of the positive CD band around 280 nm, but the overall features of the CD spectrum remained unchanged indicating a retained B-type conformation. The increase of the positive band was larger (Fig. 7) and occurred slightly faster (Fig. 8) than for the complex with B-form poly(dG-m^{5}dC) (Fig. 5).

With both the B- and Z-conformations of poly(dG-dC), the RecA association rates were faster than for the binding to methylated poly(dG-dC) observed above (compare Figs. 5 and 8). The half-times of the reactions were less than 15 min for both conformations. The difference in binding rates between the two forms was much smaller than with poly(dG-m^{5}dC), although the binding to the Z-form appeared to be slightly slower (Fig. 8).

LD measurements were carried out to quantify the binding and to follow more accurately the association kinetics. The LD signal of free Z-form of poly(dG-dC) was twice that of the B-form (Fig. 9), which is the same as with poly(dG-m^{5}dC) (Fig. 3), indicating a proper double-helical structure (33, 35, 36). For the B-form of poly(dG-dC) the LD at 260 nm increased about 20 times upon addition of RecA (Fig. 9), and the time dependence was similar to that observed in the CD measurements (Fig. 8). LD measurements of various amounts of RecA added to 25 μM of poly(dG-dC) in B-form revealed a stoichiometry of 2.8 base pairs/RecA (Fig. 10). The magnitude of the LD at 260 nm initially increased linearly with increasing RecA concentration and reached eventually a plateau. By contrast, the LD of the Z-form decreased upon addition of RecA and virtually vanished (Fig. 9). The determination of binding stoichiometry and binding rates from LD was therefore not possible, because the LD signal vanished before complete saturation occurred. The reason for the vanishing LD is at present unclear, but it seems likely that the complex aggregates and therefore does not orient in the flow field. Mn^{2+}-induced Z-DNA has been reported to be prone to aggregation (34). Under low salt conditions, also the formation of RecA B-DNA complex is associated with increased turbidity indicating a tendency of aggregation (17).

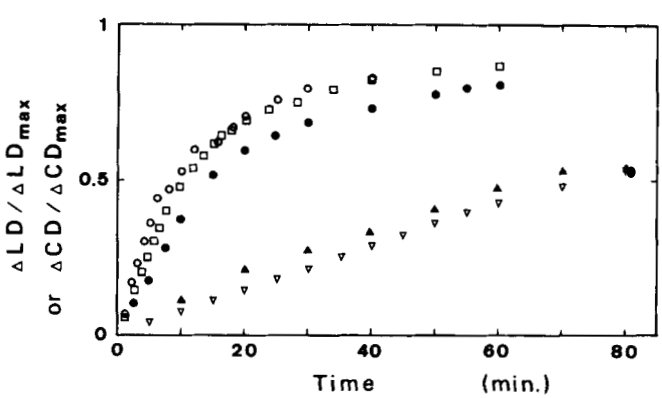


FIG. 8. **Association kinetics of RecA binding to B- and Z-forms of poly(dG-dC).** Relative changes in CD and LD with time expressed as in Fig. 5. Changes in CD (○) and LD (□) after addition of RecA to B-form in 2.5 mM $MnCl_2$ and change in CD (●) after addition to Z-form in 2.5 mM $MnCl_2$. Changes in CD after addition of RecA to B-form poly(dG-dC) in 2.5 mM $MgCl_2$ with (▲) and without (▽) preheating. Same conditions as in Fig. 7.

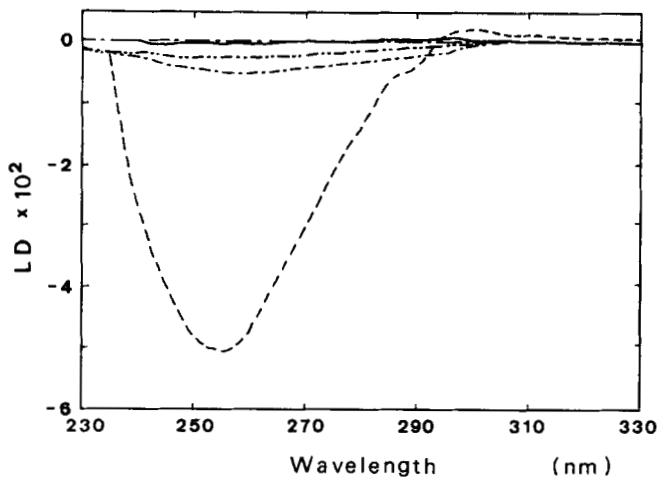


FIG. 9. **LD spectra of RecA•poly(dG-dC) complexes.** LD of RecA complex with B- (– – –) and Z-form (—) of poly(dG-dC) in 2.5 mM $MnCl_2$ and of free B- (– ·· –) and Z (– · –)-form of free polynucleotide. Same conditions as in Fig. 7. Shear gradient 600 s^{-1}.

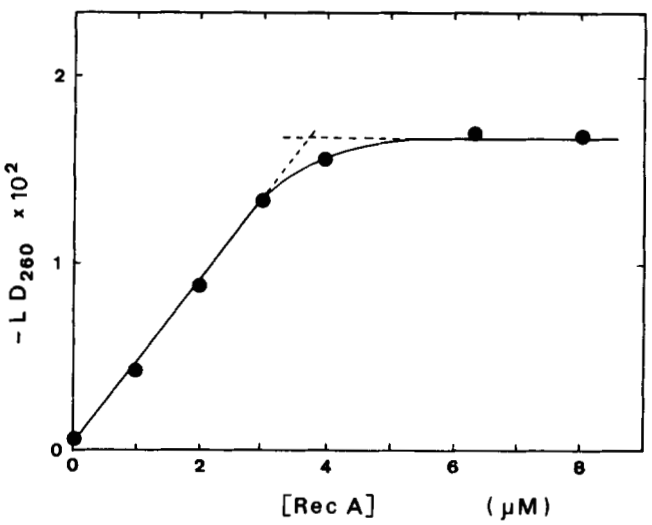


FIG. 10. **Determination of binding stoichiometry of RecA complex with B-form poly(dG-dC).** LD at 260 nm of 25 μM poly(dG-dC) in B-form mixed with various amounts of RecA. Shear gradient 600 s^{-1}. Same conditions as in Fig. 7*b*.

To examine whether heating the poly(dG-dC) has any effects on the complex formation, we compared the binding of RecA to the polynucleotide before and after heating to 60 °C in the presence of 2.5 mM $MgCl_2$ instead of $MnCl_2$. In absence of Mn^{2+}, poly(dG-dC) remains in the B-form even after heating (Fig. 7*c*). In both cases a slow increase of the CD at 280 nm was observed upon addition of RecA (Fig. 8). The changes in CD spectrum (Fig. 7*c*) were smaller than in the presence of $MnCl_2$ (Fig. 7*b*) and the binding rates much slower (Fig. 8): the half-time was more than 60 min with both the heated and non-heated polynucleotide, and the LD at 260 nm increased about 20 times in magnitude upon RecA binding (not shown). These results indicate that heating the polynucleotides has no significant effect on RecA binding, whereas the nature of the metal ion clearly has.

Addition of NaCl to the free Z-form of poly(dG-dC) transforms it back to the B-form, as judged from the progressive decrease of the CD at 270 nm (results not shown). Complete

transformation back to B-form was obtained in about 140 mM NaCl, and the CD spectrum was similar to that of the B-form of poly(dG-dC) in absence of $MnCl_2$. The Z-form of poly(dG-dC) complexed with RecA could also be transformed back to the B-form by addition of NaCl (not shown). This shows that the binding of RecA does not stabilize the $MnCl_2$-induced Z-conformation of poly(dG-dC) either. After the retransformation the majority of the RecA remained bound to DNA as judged from CD.

DISCUSSION

We have investigated the interaction of RecA with Z-form of DNA in terms of binding stoichiometry and association kinetics using polarized light spectroscopy. These results are compared with the RecA binding to the same DNA in B-form under else identical or similar conditions. In addition to stoichiometric and reaction-mechanistic information, the spectroscopic measurements have also provided structural information about the RecA·DNA complexes.

Structure of RecA·Z-DNA Complexes

Conformation of Polynucleotide in RecA·DNA Complexes—Circular dichroism measurements reveal that the binding of RecA does not significantly alter the conformation of the polynucleotide (Figs. 1 and 7): Z-form DNA remains in the Z-form and B-form DNA remains in the B-form after the binding of RecA. In no case was it observed that binding of RecA induces conformational changes of the polynucleotides, and it is concluded that the conformation of the polynucleotide in solution is preserved upon RecA binding.

Differences in Global Structure of Complexes with Z- and B-form DNA—LD spectra of RecA complexes with B- and Z-form DNA exhibit similar features: a negative band centered at 260 nm and a small positive band above 290 nm partly overlapping the negative DNA LD. The LD reflects the orientation of the prominent in-plane transition moments of the light-absorbing DNA base and tryptophan chromophores relative to a preferred orientation axis of the complex (12). This indicates, in both types of complexes, a perpendicular orientation of the DNA bases and a more parallel orientation of the aromatic planes of the tryptophan residues in RecA relative to the fiber axis of the complex. The binding stoichiometry is similar in all complexes: about 3 base pairs/RecA monomer, possibly slightly larger for the complex with Z-DNA. The organization of the DNA and the proteins appears thus to be similar in the two types of complexes, *i.e.* very probably a helical arrangement of RecA also around Z-form DNA as indicated for B-DNA (6–9).

The amplitude of the LD for the RecA complex with Z-form of poly(dG-m^5dC) is, somewhat surprisingly, considerably smaller than with the B-form (Fig. 3). For the complex with the Z-form of poly(dG-dC) induced by Mn^{2+} the LD signal virtually vanishes (Fig. 9). This could indicate that the RecA complex with Z-form DNA is more flexible or more condensed (or aggregated) than the complex with B-form DNA, despite the fact that free Z-DNA is stiffer than free B-DNA (33). The difference may also be due to less perpendicular DNA base orientation in the Z-form complex.

Association Kinetics

The association of RecA to double-stranded DNA is very slow in all cases, and the rate depends on the conformation of the DNA and on the nature of the positively charged divalent ions present. Under similar conditions and with the same DNA, the association rate to Z-form DNA is always slower than to the B-form (Fig. 5 and 8), a finding that seems to be in conflict with the conclusions of Blaho and Wells (22).

Their conclusions were mainly based on the comparison of the binding of RecA to unmodified poly(dG-dC) in B-form and to the modified polynucleotides poly(dG-m^5dC) and poly(dG-BrdC) in Z-form. However, it cannot be excluded that the modification of the DNA bases *per se* might influence the binding rates of RecA (13, 15, 21). When we compared the binding of RecA to unmodified poly(dG-dC) with the binding to the modified polynucleotide poly(dG-m^5dC) under identical conditions (2.5 mM $MgCl_2$, compare Figs. 5 and 8), we found the association to the modified polynucleotide to be significantly faster irrespective of the nucleotide conformation. Because the lengths of the polynucleotides were not necessarily the same this comparison is not strictly justifiable, but our results might explain the observations of Blaho and Wells (22), and we conclude that modification of the DNA bases influences the association rates. A preferential binding of RecA to modified double-stranded DNA has also been observed previously both *in vivo* and *in vitro*: RecA binds more tightly and quickly to DNA modified by UV irradiation (21) or by the carcinogen *N*-2-acetylaminofluorene.[3,4]

We find the RecA binding to poly(dG-dC) in the presence of Mn^{2+} to be much faster than in presence of Mg^{2+}, as also reported by Blaho and Wells (22), and to depend much less on the conformation of the polynucleotide. However, Blaho and Wells observed in a filter binding assay (22) a slightly larger retention of the RecA complex with Z-form DNA than of the complex with the B-form, in contrast to the slightly slower binding of RecA to the Z-form of DNA reported here. This discrepancy might be due to a difference in the efficiency of retention or binding cooperativity of the complexes with Z- and B-form DNA. The filter binding assay is known to be influenced by other factors than binding affinity (37, 38). Another possibility might be partial denaturation of poly(dG-dC) caused by the heating done to induce the Z-form. Blaho and Wells (22) heated the poly(dG-dC) in the absence of NaCl, whereas we induced the Z-form in the presence of 40 mM NaCl. The higher ionic strength should increase the melting temperature and prevent strand separation. Even in 40 mM NaCl heating to above 70 °C followed by progressive cooling induces a certain degree of non-double helical structure, as judged from a decrease of the LD signal (results not shown), to which the binding of RecA is faster. Finally, it should be recalled that our measurements, in contrast to those of Blaho and Wells (22), were carried out in the presence of ATPγS which does not hydrolyze. In fact, they observed considerable lags in ATP hydrolysis rates with Z-DNA compared to B-DNA.

From these results it is concluded that the binding rate of RecA to double-stranded DNA (in presence of ATPγS) depends both on conformation and chemical modification of the DNA, as well as on the nature of the metal cations. RecA binds faster to DNA in B-conformation than in Z-conformation (Figs. 5 and 8), and faster to methylated poly(dG-dC) than to unmodified poly(dG-dC) (Figs. 5 and 8) and also faster in the presence of Mn^{2+} compared to Mg^{2+} (Fig. 8).

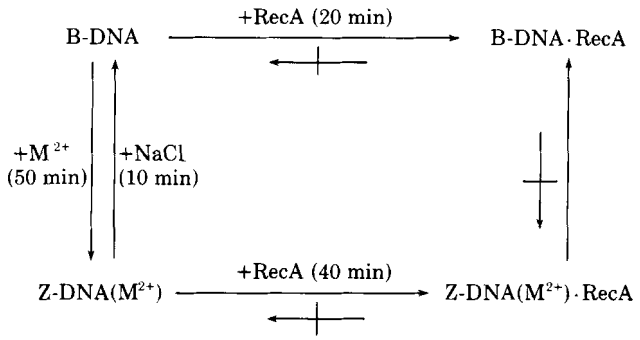
Stability of RecA·DNA Complexes

For both poly(dG-dC) and poly(dG-m^5dC) our conclusions regarding the relative stability of the RecA complexes with the polynucleotides in their B- and Z-conformational states

[3] N. Koffel-Schwartz and R. Fuchs, personal communication.

[4] X. Veaute, I. Bertrand, M. Takahashi, and R. Fuchs, unpublished results.

can be summarized by the following reaction scheme ($\tau_{1/2}$ is given in parentheses for the Mg^{2+}-induced Z-form of poly(dG-m^5dC)).



SCHEME 1

The RecA-DNA binding is characterized by a very high stability constant (3, 4), and the binding reaction can therefore be considered as practically irreversible. This is important to recall when considering the NaCl-induced retransformation of the B-form from the Z-form of polynucleotides in complex with RecA. Although we known as yet very little about the structural details of these complexes, it is not difficult to imagine how the compactly stacked array of RecA proteins may provide a (kinetic) barrier to major conformational changes in the DNA double helix. Thus, it is not astonishing that B-form poly(dG-dC), when complexed with RecA, is prevented from undergoing a transition to Z-form. What is more surprising, however, is the observation that divalent metal-induced Z-form is retransformed to B-form by addition of NaCl, also when associated with RecA. Furthermore, this reaction occurs at practically the same Na^+ concentration and with the same kinetics as in the absence of RecA, demonstrating that the protein interaction has no noticeable stabilizing effect on the Z-conformation.

Implications on Models for Recombination Mechanism

It has been speculated that one DNA molecule is in a left-handed (Z-type) configuration in the genetic recombination (23). Our results clearly demonstrate that *in vitro* the DNA conformation is retained upon RecA binding and that only Z → B, but not the B → Z, conformational transition may occur in the RecA·DNA complex. We therefore suggest that if the Z-conformation is indeed involved in the *in vivo* recombination, the DNA should be in the Z-conformation prior to the RecA association. Once the RecA·Z-DNA complex is formed, the RecA should not prevent transformation to the B-form when the physical constraints disappear, for example after the cleavage of DNA.

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