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Binding of RecA protein to single-stranded nucleic acids: spectroscopic studies using fluorescent polynucleotides

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Binding of the *recA* gene product from *Escherichia coli* to single-stranded polynucleotides has been investigated using poly(dA) that have been modified by chloroacetaldehyde to yield fluorescent 1,N^6-ethenoadenine (ϵA) bases. A strong enhancement of the fluorescent quantum yield of poly(dϵA) is induced upon RecA protein binding. A 4-fold increase is observed in the absence of ATP or ATPγS and a 7-fold increase in the presence of either nucleoside triphosphate. RecA protein can bind to poly(dϵA) in the absence of both Mg^{2+} ions and ATP (or ATPγS) but Mg^{2+} ions are required to observe RecA protein binding in the presence of ATP (or ATPγS) at pH 7.5. ATP binding to the RecA-poly(dϵA) complex induces a dissociation of RecA from the polynucleotide followed by re-binding of [RecA-ATP-Mg^{2+}] ternary complex. Whereas ATP-induced dissociation of RecA-poly(dϵA) complexes is a fast process, the subsequent binding reaction of [RecA-ATP-Mg^{2+}] is slow. A model is proposed whereby [RecA-ATP-Mg^{2+}] binding to poly(dϵA) involves slow nucleation and elongation processes along the polynucleotide backbone. The nucleation reaction is shown to involve at least a trimer or a tetramer. Polymerization of the [RecA-ATP-Mg^{2+}] ternary complex stops when the polynucleotide is entirely covered with 6 ± 1 nucleotides per RecA monomer. ATP hydrolysis then induces a release of RecA-ADP complexes from the polynucleotide template.

Key words: RecA protein/*Escherichia coli*/polynucleotides/fluorescence

Introduction

The *recA* gene product from *Escherichia coli* is involved in two very important processes: general genetic recombination and DNA repair. In relation to its role in genetic recombination, RecA protein catalyzes several *in vitro* reactions such as annealing of two complementary single strands (Weinstock *et al.*, 1979), assimilation of linear single strands into duplex DNA (D-loop formation) (Shibata *et al.*, 1979; McEntee *et al.*, 1979), pairing of gapped circular double-stranded DNA with either superhelical or nicked circular DNA (West *et al.*, 1980; Cunningham *et al.*, 1980) and complete strand exchange between linear duplex DNA and homologous circular single-stranded DNA (ssDNA) (Das Gupta *et al.*, 1980; West *et al.*, 1981a, 1981b; Cox and Lehman, 1981). In all these reactions, RecA protein must bind both single-stranded and double-stranded DNA. ATP binding is required for D-loop formation but not its hydrolysis as ATPγS, a non-hydrolyzable analog of ATP, can substitute. However, ATP hydrolysis is required for branch migration that proceeds with a unique polarity (Cox and Lehman, 1981).

The *recA* gene product plays an important role in post-replication DNA repair processes that involve recombination steps. In addition, when the replication fork is blocked, the RecA protein is activated as a protease that cleaves the *lexA* gene product. The latter is the repressor of a series of genes which are involved in DNA repair *(uvrA, B)*, in recombination *(recA, himA)*, in filamentation *(sfiA)*, in mutagenesis *(umuC)* and in other unknown functions (*din* genes). It also controls its own expression at the transcriptional level. Cleavage of LexA repressor by the RecA protease leads to the derepression of all genes that are under *lexA* control and this phenomenon is known as the 'SOS response' (Little and Mount, 1982). The *recA* protease also cleaves the λ repressor leading to λ prophage induction in lysogenic strains. The proteolytic activity of RecA protein is induced when it binds to ssDNA in the presence of ATP even though ATP hydrolysis is not required (ATPγS can substitute) (Craig and Roberts, 1980).

To understand better how the two enzymatic activities of RecA protein (ATPase and protease) are stimulated, it is necessary to have more knowledge of its DNA binding properties with respect to both single-stranded and duplex structures. We previously showed that the intrinsic fluorescence of the RecA protein tryptophyl residues was quenched upon binding to poly(dT), heat-denatured DNA and single-stranded fdDNA (Hélène *et al.*, 1982). However, fluorescence quenching was very weak and could not be used easily to investigate the effects of different ligands. Also, the apparent stoichiometry of RecA binding to ssDNA, deduced from fluorescence quenching experiments, did not agree with that determined from the concentration dependence of RecA enzymatic activities. In this paper we report the results of experiments carried out using a fluorescent polynucleotide, poly(dϵA), obtained by reacting poly(dA) with chloroacetaldehyde. Binding of RecA protein to this polynucleotide strongly enhances its fluorescence quantum yield; this can be conveniently utilized to analyze the requirements for RecA protein-DNA binding. Some experiments were also carried out with ϵ-DNA, the product of chloroacetaldehyde modification of heat-denatured *E. coli* DNA. Both adenine and cytosine bases are modified but only ϵ-adenine emits fluorescence at neutral pH (Barrio *et al.*, 1972). The reaction with chloroacetaldehyde was carried out until 100% of adenine bases had reacted both in poly(dA) and in denatured DNA. The fluorescence of ϵDNA has already been reported to be enhanced upon RecA protein binding (Silver and Fersht, 1982, 1983).

Results

Fluorescence changes induced by RecA protein binding to poly(dϵA)

The fluorescence emitted by poly(dϵA) at 20°C in buffer F (20 mM Tris HCl, 4 mM $MgCl_2$, 1 mM β-mercaptoethanol, 0.1 mM EDTA, pH 7.5) is characterized by a broad band whose maximum is ~410 nm. Excitation was performed at 305 nm to minimize absorption of exciting light by RecA pro-

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tein. The small contribution due to the fluorescence of RecA protein at 410 nm under 305 nm excitation was taken into account and subtracted from that of the RecA-poly(dεA) mixtures.

Addition of RecA protein to poly(dεA) in buffer F at 20°C leads to an enhancement of the εA fluorescence intensity (Figure 1). At low RecA/poly(dεA) ratios the fluorescence intensity increases linearly with RecA protein concentration and then levels off. Extrapolation of the linear part gives a stoichiometric ratio of one RecA monomer per 6 ± 1 bases. The same value was obtained when the poly(dεA) concentrtaion was increased 4-fold indicating that RecA protein binds stoichiometrically to the polynucleotide under our experimental conditions.

In the presence of ATPγS the fluorescence increase induced by RecA binding to poly(dεA) is larger than in the absence of ATPγS (Figure 1). The fluorescence intensity reached when the polynucleotide is fully covered by RecA protein is 7-fold higher than that of free poly(dεA) in the presence of ATPγS. It is only 4-fold higher in the absence of ATPγS (Figure 1). Similar results were obtained when ATPγS was replaced by ATP. Qualitatively similar results were recently reported by Silver and Fersht (1982) with εDNA. Using an εDNA sample in which all adenine and cytosine bases have been converted to εA and εC by reaction with chloroacetaldehyde we have obtained fluorescence enhancement factors of 2.7 and 3.3 in the absence and in the presence of 0.1 mM ATPγS, respectively.

Using ^{3}H-labelled ATP, it was shown that poly(dεA) was able to support RecA-mediated ATP hydrolysis. The initial velocity of the hydrolysis reaction increased with the poly(dεA)/RecA ratio until one RecA monomer was bound per 6 ± 1 bases, in agreement with the stoichiometry determined above for the binding of the protein to poly(dεA) and with previous results using ssDNA to stimulate the ATPase activity of RecA protein (Craig and Roberts, 1980; Weinstock *et al.*, 1981). Competition experiments were carried out to compare the affinity of RecA protein for poly(dA) and poly(dεA). RecA protein was added to a mixture of these two polynucleotides and the increase of poly(dεA) fluorescence was recorded. The same curve was obtained whether poly(dεA) was used alone or in an equimolar mixture with poly(dA). Even when the concentration of poly(dA) was 10 times higher than that of poly(dεA) the fluorescence increase was identical to that observed in the absence of poly(dA). From this absence of competition it can be concluded that RecA protein binding to poly(dεA) is much tighter (at least 100 times) than that to poly(dA). Therefore adenine modification by chloroacetaldehyde to yield ε-adenosine strongly enhances RecA protein binding. A similar conclusion was reached when εDNA was compared with unmodified ssDNA (unpublished results; Silver and Fersht, 1983).

Kinetics aspects

Binding of RecA protein to poly(dεA) in the absence of ATPγS is completed within the 10 s required to mix the protein and polynucleotide solutions and to reach temperature equilibrium. On the contrary, a slow process is observed in the presence of ATPγS (Figure 2). It should be noted that even though the final fluorescence level is higher in the presence of ATPγS, the fluorescence intensity 10 s after mixing is lower than in the absence of ATPγS.

The slow process can itself be divided into two phases. The

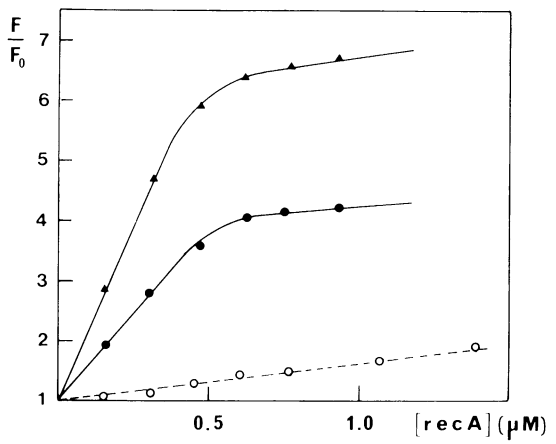

Fig. 1. Change in relative fluorescence intensity of poly(dεA) (3.8 μM) upon addition of RecA protein in the absence (●) and in the presence (▲) of 88 μM ATPγS and in the presence of 1.4 mM ADP (○). Measurements were carried out at 20°C in buffer F, pH 7.5, containing 20 mM Tris-HCl, 4 mM $MgCl_2$, 1 mM 2-mercaptoethanol and 0.1 mM EDTA. Excitation at 305 nm; fluorescence measured at 410 nm. The small contribution of RecA protein to the fluorescence at 410 nm has been subtracted.

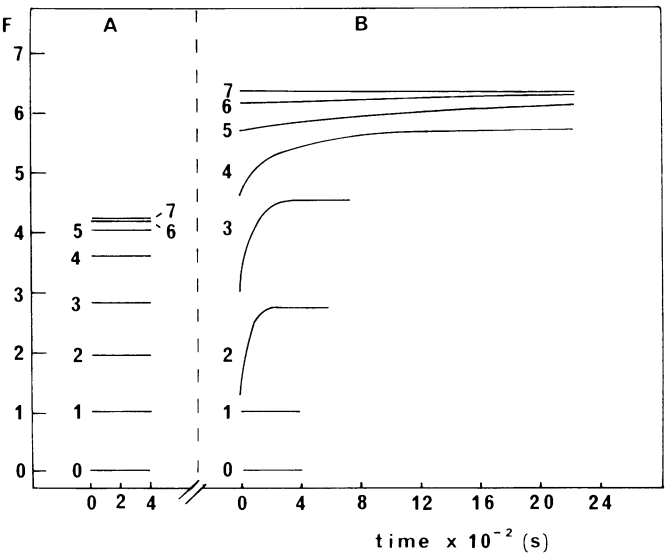

Fig. 2. Time dependence of the fluorescence of 3.8 μM poly(dεA) (λ_{exc} = 305 nm, λ_{em} = 410 nm) at different RecA protein concentrations RecA = 0 **(1)**, 0.16 μM **(2)**, 0.31 μM **(3)**, 0.47 μM **(4)**, 0.63 μM **(5)**, 0.78 μM **(6)** and 0.94 μM **(7)**. The left part **(A)** shows the results obtained in the absence of ATPγS and the right part **(B)** those obtained in the presence of 88 μM ATPγS. Experiments were carried out at 20°C in buffer F (see legend of Figure 1).

first addition of RecA protein leads to an initial step whose reaction half-time is ~50 s at 20°C. Then a slower process is observed whose time range increases when the degree of polynucleotide saturation increases. This process probably reflects the rearrangement of bound protein molecules along the polynucleotide lattice (see Discussion).

ATPγS (100 μM) was also added to a RecA-poly(dεA) complex. The fluorescence first decreased immediately after mixing and then slowly increased to reach the same final level as that obtained upon adding RecA protein to a poly(dεA) + ATPγS mixture. The same phenomenon was also observed with ATP (Figure 3) but in addition a slow decrease in the fluorescence intensity was observed at longer times. This

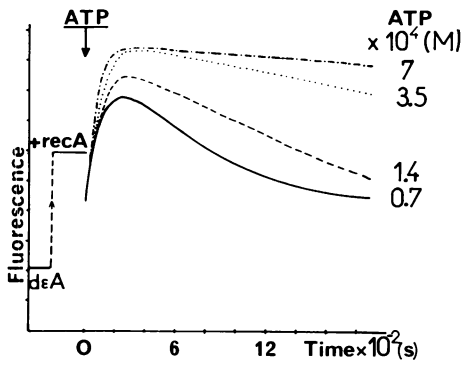

Fig. 3. Effect of ATP on the RecA-poly(dεA) complex formed in buffer F at 20°C. RecA protein (0.47 μM) was added to 3.8 μM poly(dεA) (left part). ATP was added at time 0 and the fluorescence recorded as a function of time. Different concentrations of ATP were used in separate experiments.

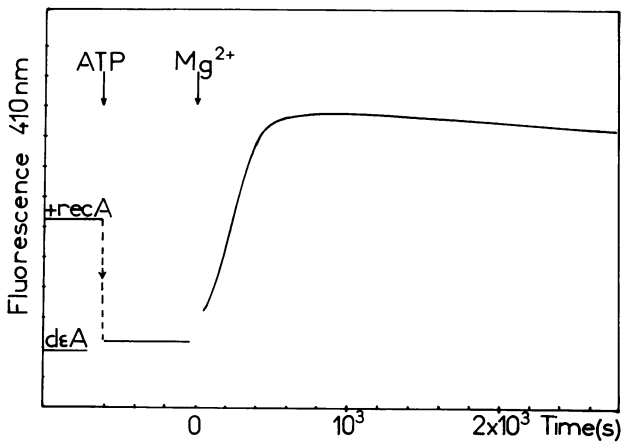

Fig. 4. Effect of ATP and Mg^{2+} ions on the RecA-poly(dεA) complex formed in buffer F' (buffer F without 4 mM $MgCl_2$). RecA protein (0.47 μM) was added to poly(dεA) (3.8 μM). Then 0.7 mM ATP and 4 mM $MgCl_2$ were successively added to the complex and fluorescence recorded as a function of time. Time 0 corresponds to the addition of $MgCl_2$. The difference between the fluorescence intensities of poly(dεA) alone and the poly(dεA) + RecA protein + ATP mixture is due to the small contribution of RecA protein to fluorescence emission at 410 nm. Mg^{2+} slightly enhances the fluorescence of poly(dεA).

decrease depends on ATP concentration and occurs in the same time range as the hydrolysis of ATP to ADP measured using ^{3}H-labelled ATP. ADP is known to bind RecA protein with nearly the same affinity as ATP in the absence of ssDNA. This binding is reduced approximately 3-fold in the presence of ssDNA (Cotterill *et al.*, 1982). It was therefore necessary to know whether the fluorescence intensity decrease observed upon ATP hydrolysis was due either to a dissociation of RecA protein from the polynucleotide or to a change in conformation of the complex formed with poly(dεA) occurring upon conversion of ATP to ADP without dissociation of the RecA-ADP complex. In Figure 1 it is shown that adding RecA protein to poly(dεA) in the presence of a saturating concentration of ADP (1.4 mM) led to a slight linear increase in poly(dεA) fluorescence intensity. No plateau was reached even when the RecA/poly(dεA) ratio was as high as one RecA monomer per two nucleotides. These results show that the RecA-ADP complex still retains some capacity to bind to poly(dεA) at low ionic strength but that binding is very weak compared with that of the RecA-ATP complex. Therefore the slow fluorescence decrease observed at long times after adding ATP to the RecA-poly(dεA) complex (Figure 3) is due to the release of the RecA-ADP complex from the polynucleotide.

Similar conclusions can be reached from a study of light scattering. Binding of RecA protein to poly(dεA) in buffer F leads to an increase in scattered light intensity. Adding ATP or ATPγS first induces a decrease in light scattering followed by a slow increase. Hydrolysis of ATP is then accompanied by a decrease of the scattering intensity (results not shown).

Effect of Mg^{2+} ions

All the experiments described above were carried out in buffer F containing 4 mM $MgCl_2$. To obtain more information on the role played by Mg^{2+} ions a series of experiments were performed in buffer F' containing 20 mM Tris-HCl, 0.1 mM EDTA, 1 mM β-mercaptoethanol. As shown in Figure 4, RecA protein was able to bind to poly(dεA) in the absence of Mg^{2+} ions and it induced nearly the same final fluorescence increase as in buffer F containing 4 mM $MgCl_2$. The binding curve exhibits a sigmoidal shape characteristic of cooperative binding. A similar shape was observed in the presence of Mg^{2+} ions at ionic strengths higher than 0.1 M NaCl; at lower ionic strengths (buffer F) binding is probably too strong for the sigmoidal curve to be observed. These results indicate that RecA protein binding to poly(dεA) is cooperative under all conditions tested (Cazenave *et al.*, in preparation).

Addition of ATP (0.7 mM) or ATPγS (0.1 mM) led to a reversal of the fluorescence enhancement due to RecA protein binding to poly(dεA). Further addition of 4 mM $MgCl_2$ to the RecA/poly(dεA)/ATP(ATPγS) mixture induced a time-dependent increase in poly(dεA) fluorescence with the same kinetics as that observed when ATP (ATPγS) was added to the RecA-poly(dεA) complex formed in the presence of 4 mM $MgCl_2$ (see Figure 3). Hydrolysis of ATP was then accompanied by a slow decrease of poly(dεA) fluorescence as described above.

The first addition of RecA protein to poly(dεA) in the absence of Mg^{2+} ions leads to an initial increase of εA fluorescence which is higher than the final equilibrium level. This might be due to the higher level of aggregation of RecA protein in the conservation buffer as compared with that in the binding buffer, especially as a result of the higher concentration. As will be discussed, aggregation of RecA protein is an important factor in its DNA binding properties.

Discussion

The use of a fluorescent polynucleotide, poly(dεA), has made it possible to obtain important information on the mode of RecA protein binding to single-stranded polynucleotides. Binding of RecA protein to poly(dεA) enhances the fluorescence quantum yield of εA bases 4-fold and 7-fold in the absence and the presence of ATP (ATPγS), respectively. The fluorescence of ε-adenine is very sensitive to stacking interactions which induce a strong fluorescence quenching (Tolman *et al.*, 1974; Baker *et al.*, 1978). It is therefore very likely that the fluorescence increase observed upon RecA binding to poly(dεA) is due to unstacking of the bases. It should be noted that the fluorescence quantum yield of poly(dεA) is ~12 times lower than that of the isolated nucleotide, as determined from hydrolysis experiments. Therefore, a 4-fold or

7-fold increase is still far from that corresponding to a completely stretched and unstacked polynucleotide. The same stoichiometry (6 ± 1 nucleotides per RecA monomer) is obtained independently of whether ATP or ATPγS is present or not. This result means that the number of nucleotide units covered by one RecA monomer is quite similar in both cases.

The fact that both ϵDNA and poly(dϵA) are able to stimulate the ATPase activity of RecA protein and that the binding site size is similar to that obtained with other single-stranded polynucleotides [poly(dT) or poly(dU), for example] strongly suggests that the complexes formed with these modified nucleic acids are similar to those formed with any single-stranded DNA even though replacing adenine by ϵ-adenine does increase the binding strength and the ATPase activity of RecA protein. It should be noted that poly(rϵA) does not bind RecA protein (Silver and Fersht, 1982) and does not stimulate ATPase activity (unpublished results) in agreement with the known specificity of this protein for polydeoxynucleotides.

Adding ATP (ATPγS) to the RecA-poly(dϵA) complex first leads to a rapid dissociation of RecA protein from its polynucleotide binding sites both in the presence (Figure 3) and in the absence (Figure 4) of Mg^{2+} ions. This is demonstrated by the reversal of the fluorescence enhancement induced by RecA protein binding to poly(dϵA) in the absence of ATP (ATPγS) and by a decrease of the intensity of light scattering. In the absence of Mg^{2+} ions the system remains in its dissociated state. On the contrary, in the presence of Mg^{2+} ions, RecA protein binds again to poly(dϵA) in a very slow reaction. The kinetics of this process appears to involve at least two phases: initial binding with a half-reaction time of ~50 s at 20°C followed by a very slow process. The latter is probably due to a redistribution of bound RecA molecules. It is accompanied by a slight increase in fluorescence intensity indicating that the redistribution of bound RecA molecules allows for some additional binding of free molecules. This kind of rearrangement has already been observed with several protein-nucleic acid complexes (Lohman and Kovalczykowski, 1981).

Since both ATP (ATPγS) and Mg^{2+} ions are required to observe the slow binding process it seems reasonable to assume that the slow binding species is a ternary complex RecA-ATP-Mg^{2+}. However the binding of either ATP (ATPγS) or Mg^{2+} ions is expected to be a diffusion-controlled process and the formation of the ternary system is not likely to be the rate-limiting step which could explain the slow kinetics of binding. Several hypotheses can be put forward: (i) simultaneous or successive binding of ATP and Mg^{2+} ions induces a slow conformational change in the RecA protein, (ii) the ternary complex RecA-ATP-Mg^{2+} undergoes a self-association reaction and the binding species is an oligomer [RecA-ATP-Mg^{2+}]$_m$. The nucleation process would then be followed by an elongation reaction along the polynucleotide matrix to give a RecA polymer, (iii) nucleation and polymerization both occur on the polynucleotide matrix, i.e., oligomeric species [RecA-ATP-Mg^{2+}]$_m$ form upon RecA-ATP-Mg^{2+} binding to the nucleic acid.

Preliminary experiments using oligodeoxyadenylates modified by chloroacetaldehyde indicate that a minimum length of the oligonucleotide is required to observe RecA binding both in the absence and presence of ATPγS. No binding was observed with $(d\epsilon A)_8$, $(d\epsilon A)_{12}$ and $(d\epsilon A)_{18}$. Binding starts with $(d\epsilon A)_{19-24}$ a mixture of oligodeoxynucleotides whose lengths vary between 19 and 24 monomer units. Since we have determined a site size of 6 ± 1 nucleotides per RecA monomer these results indicate that the binding species is at least a trimer or a tetramer. The Hill coefficient for the binding of ATP or ATPγS to a RecA-ϵDNA complex has been determined from the fluorescence enhancement of ϵDNA (Silver and Fersht, 1982). Values ranging from 2.4 to 4.9 have been found depending on the initial concentrations of reactants. These results demonstrate that ATP converts the RecA-ϵDNA complex into a RecA-ATP-Mg^{2+}-ϵDNA complex in a highly cooperative process. The DNA-dependent ATPase activity of RecA protein also exhibits a high degree of cooperativity with respect to ATP (Weinstock *et al.*, 1981).

Why should RecA protein first dissociate from the polynucleotide matrix upon addition of ATP in order to reassociate as a RecA-ATP-Mg^{2+} complex? The explanation might be found in the structural rearrangement of the oligomeric species which starts the nucleation process. It might be easier for the protein oligomer to rearrange its structure (upon ATP and Mg^{2+} binding) while free in solution rather than bound to the polynucleotide backbone. This probably reflects the fact that the organization of RecA monomers in the polynucleotide complex formed in the absence of ATP is not identical to that of the RecA-ATP-Mg^{2+} ternary complex. However incubation of RecA protein with ATP (ATPγS) and Mg^{2+} ions for 1 h before adding poly(dϵA) does not accelerate the binding reaction. This indicates that the nucleation reaction takes place on the polynucleotide matrix. Binding of the RecA-ATP-Mg^{2+} ternary complex to ϵDNA is faster than with poly(dϵA). Since ϵDNA binds RecA protein with a higher affinity than poly(dϵA) (unpublished results) this suggests that the rate of the nucleation reaction depends on the affinity of RecA protein for its nucleic acid sites and that nucleation is the rate-limiting step in RecA polymerization along the polynucleotide matrix.

These properties of RecA protein binding in the presence of ATP and Mg^{2+} are reminiscent of several proteins which spontaneously polymerize in the presence of an effector such as ATP (actin) (Korn, 1982) or GTP (tubulin) (Carlier, 1982) or which require a nucleic acid matrix to polymerize such as the tobacco mosaic virus (TMV) protein (Hirth and Richards, 1981; Butler and Lomonossoff, 1980).

The slow kinetic processes that we have described above for the binding of RecA protein to poly(dϵA) have been observed with both ATP and ATPγS as effectors. RecA protein binds ATP and ATPγS in the absence of Mg^{2+} ions; it also binds to both single-stranded and double-stranded DNA under the same conditions. Magnesium ions bind to both RecA protein and the RecA-ATP(ATPγS) binary complex although we cannot exclude: (i) that ATP-Mg^{2+} is the actual ligand of RecA protein, with Mg^{2+} either binding to ATP molecules already bound to RecA protein or inducing a dissociation of ATP followed by re-binding as an ATP-Mg^{2+} species and (ii) that Mg^{2+} plays a role at the level of the polynucleotide structure. Mg^{2+} ions are required to induce the DNA-dependent ATPase activity of RecA protein. However this might be related to the absolute requirement of Mg^{2+} ions to allow RecA protein binding to ssDNA in the presence of ATP rather than implying Mg^{2+} ions in the hydrolysis reaction itself. Hydrolysis of ATP leads to a dissociation of RecA protein from the polynucleotide matrix. This is due to the weak affinity of the RecA-ADP-Mg^{2+} complex for single-stranded nucleic acids. Dissociation does not occur

when ATP is replaced by ATPγS in agreement with the observation that this ATP analog is not hydrolyzed by RecA protein. Further experiments are clearly required to clarify all the kinetic steps involved in the binding of RecA protein to nucleic acids.

Using a fluorescent polynucleotide has allowed us to show that both ATP and Mg^{2+} ions are simultaneously required to observe the formation of the biologically-important complex involved in genetic recombination and DNA repair and that the binding of the [RecA-ATP-Mg^{2+}] ternary complex involves a nucleation process followed by an elongation of RecA polymers along the polynucleotide backbone. It may be speculated that once the nucleation-elongation process has been started then polymerization will proceed along any nucleic acid structure. Thus it should be possible for RecA protein to 'invade' double-stranded structures if nucleation has taken place on a single-stranded fragment of sufficient length ($n > 20$). This might explain (i) the dissociation of D-loops by excess RecA protein and the consequent 'inactivation' of circular double-stranded DNA (Shibata *et al.*, 1982) and (ii) the high ATPase activity of RecA protein stimulated by double-stranded DNA containing single-stranded gaps when these gaps are long enough ($\simeq 30$ nucleotides) to start the nucleation process (West *et al.*, 1980).

Materials and methods

RecA purification

The *recA* protein was purified from strain KM1842, kindly given to us by Dr.K.McEntee (Stanford University, California University), according to the procedure of Cox *et al.* (1981). The protein was >98% pure as judged by SDS-polyacrylamide gel electrophoresis. It was tested for its ssDNA-dependent ATPase activity as described by Weinstock *et al.* (1981). Our preparation hydrolyzed 12 mol ATP per *recA* monomer per min at 37°C in 20 mM Tris HCl pH 7.5, 10 mM $MgCl_2$, 20 mM NaCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol. Protein concentration was determined from its absorbance at 280 nm using $E_{280}^{1\%} = 5.16$ (Cox *et al.*, 1981).

Preparation of the ethenoderivatives of polynucleotides

Heat-denatured calf thymus DNA and poly(dA) were modified by chloroacetaldehyde according to Kohwi-Shigematsu *et al.* (1978). Polynucleotides, at a concentration between 4 and 8 x 10^{-4} M (expressed in mol of nucleotides) were incubated for 48 h at 37°C with 0.19 M chloroacetaldehyde in a 20 mM sodium phosphate buffer pH 6.8, and extensively dialyzed against 20 mM Tris HCl pH 7.5 to remove chloroacetaldehyde prior to use. H.p.l.c. of the hydrolysis products of the modified polynucleotides after total enzymic digestion by snake venom phosphodiesterase and pancreatic DNase I has shown that, in these conditions, 100% of the adenine and cytosine residues were converted to their ethenoderivatives. The polynucleotide concentration was determined from the amount of dεAMP residues liberated after total enzyme digestion. This was done by measuring the fluorescence intensity at 410 nm (λ_{exc} = 305 nm) of the hydrolysate and comparing it with the emission of an εAMP standard solution.

Calf thymus DNA was purchased from Sigma, poly(dA) from PL Biochemicals and εAMP, ATP, ADP, ATPγS from Boehringer Mannheim. Chloroacetaldehyde was a Fluka product. Fluorescence measurements were performed on an AMINCO SPF 500 spectrofluorimeter in 1 x 1 cm quartz cuvettes thermostated at 20°C.

In most of the fluorescence experiments a pH 7.5 buffer was used (referred to as buffer F). It contained 20 mM Tris-HCl, 4 mM $MgCl_2$, 1 mM β-mercaptoethanol, 0.1 mM EDTA. In some cases $MgCl_2$ was removed (see text) and the corresponding buffer referred to as buffer F′.

References

Baker,B.M., Vanderkooi,J. and Kallenbach,N.R. (1978) *Biopolymers,* **17**, 1361-1372.

Barrio,J.R., Secrist,J.A. and Leonard,N.J. (1972) *Biochem. Biophys. Res. Commun.,* **46**, 597-604.

Butler,P.J.G. and Lomonossoff,G.P. (1980) *Biophys. J.,* **32**, 295-312.

Carlier,M.F. (1982) *Mol. Cell. Chem.,* **47**, 97-113.

Cotterill,S.M., Satterhwait,A.C. and Fersht,A.R. (1982) *Biochemistry (Wash.),* **21**, 4332-4337.

Cox,M.M. and Lehman,I.R. (1981) *Proc. Natl. Acad. Sci. USA,* **78**, 3433-3437.

Cox,M.M., McEntee,K. and Lehman,I.R. (1981) *J. Biol. Chem.,* **256**, 4676-4678.

Craig,N.L. and Roberts,J.W. (1980) *Nature,* **283**, 26-30.

Cunningham,R.P., Das Gupta,C., Shibata,T. and Radding,C.M. (1980) *Cell,* **20**, 223-235.

Das Gupta,C., Shibata,T., Cunningham,R.P. and Radding,C.M. (1980) *Cell,* **22**, 437-446.

Hélène,C., Toulmé,J.J., Behmoaras,T. and Cazenave,C. (1982) *Biochimie,* **64**, 697-705.

Hirth,L. and Richards,K.E. (1981) *Adv. Virus Res.,* **26**, 145-199.

Kohwi-Shigematsu,T., Enomoto,T., Yamada,M.A., Nakanishi,M. and Tsuboi,M. (1978) *Proc. Natl. Acad. Sci. USA,* **75**, 4689-4693.

Korn,E.D. (1982) *Physiol. Rev.,* **62**, 672-737.

Little,J.W. and Mount,D.W. (1982) *Cell,* **29**, 11-22.

Lohman,T.M. and Kowalczykowski,S.C. (1981) *J. Mol. Biol.,* **152**, 67-109.

McEntee,K., Weinstock,G.M. and Lehman,I.R. (1979) *Proc. Natl. Acad. Sci. USA,* **76**, 2615-2619.

Shibata,T., Das Gupta,C., Cunningham,R.P. and Radding,C.M. (1979) *Proc. Natl. Acad. Sci. USA,* **76**, 1638-1642.

Shibata,T., Ohtani,T., Chang,P.K. and Ando,T. (1982) *J. Biol. Chem.,* **257**, 370-376.

Silver,M.S. and Fersht,A.R. (1982) *Biochemistry (Wash.),* **21**, 6066-6072.

Silver,M.S. and Fersht,A.R. (1983) *Biochemistry (Wash.),* **22**, 2860-2866.

Tolman,G.L., Barrio,J.R. and Leonard,N.J. (1974) *Biochemistry (Wash.),* **13**, 4869-4878.

Weinstock,G.M., McEntee,K. and Lehman,I.R. (1979) *Proc. Natl. Acad. Sci. USA,* **76**, 126-130.

Weinstock,G.M., McEntee,K. and Lehman,I.R. (1981) *J. Biol. Chem.,* **256**, 8829-8834 and 8845-8849.

West,S.C., Cassuto,E., Mursalim,J. and Howard-Flanders,P. (1980) *Proc. Natl. Acad. Sci. USA,* **77**, 2569-2573.

West,S.C., Cassuto,E. and Howard-Flanders,P. (1981a) *Proc. Natl. Acad. Sci. USA,* **78**, 2100-2104.

West,S.C., Cassuto,E. and Howard-Flanders,P. (1981b) *Nature,* **290**, 29-33.