Cobalt: an effector of *E. coli* *recA* protein activity

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ABSTRACT

Studies of cation requirements in the *recA*-catalyzed proteolysis of λ repressor and strand assimilation reactions have demonstrated that Co^{2+} significantly enhances both activities. In the presence of 4mM MgCl_2 , the optimal concentration of CoCl_2 for proteolysis was 1mM. 2mM Co^{2+} increased the rate and extent of D-loop formation as measured by membrane filtration. Cobalt did not replace Mg^{2+} for the ssDNA-dependent ATPase activity of *recA*, and did not affect the rate of hydrolysis of ATP, measured over a wide range of DNA concentrations. Cobalt did prevent the Mg-dependent ssDNA renaturation catalyzed by *recA* protein. Membrane filter binding assays established that Co^{2+} increases the affinity of *recA* protein for ssDNA with ATP, dATP, or ATP γ S as cofactors. The dissociation of *recA* protein from ssDNA-nucleoside triphosphate complex was much slower with CoCl_2 . This metal provides an excellent tool for dissecting the various activities inherent in *recA* protein.

INTRODUCTION

The *recA* gene product is a multifaceted protein involved in DNA recombination, DNA repair, and the induction of a diverse group of cellular functions (SOS functions) which become expressed in response to DNA damage or a block in chromosomal replication.

recA protein has been purified (1-4) and these preparations catalyze a number of reactions *in vitro*. Among these are: ssDNA dependent ATPase (2,4); ATP dependent renaturation of complementary ssDNA (1); assimilation of ssDNA into homologous double strand DNA to form a D-loop (3,5); formation of joint four strand structures from gapped and homologous duplex DNA (6,7); proteolysis of *lexA* protein (8); and proteolysis of phage repressors (9).

The proteolytic activity of purified *recA* protein occurs at slow rates *in vitro*. One mol of *recA* cleaves 0.41 mol of λ repressor in one hour (10). This reaction requires the presence of a nucleoside triphosphate and a polynucleotide (11). In light of the observation that *recA* protein amplification is not required for λ induction (12,13), it seems unlikely that the observed *in vitro* reaction, as performed at present, can account for the short period

of time required for a lysogen to be induced by external agents. In our search for parameters that influence the in vitro proteolytic reaction, we observed that cobalt, at specific concentrations, increased the rate of λ repressor cleavage. This is a report of the changes found to occur in the activity of recA protein with respect to DNA complex formation, hydrolysis of ATP, λ repressor cleavage and D-loop formation.

MATERIALS AND METHODS

Purification of recA protein. recA was isolated from strain KM1842, grown as described (1). The protein was purified by the method of Weinstock et al. (1), modified to include chromatography on DEAE-cellulose before and after phosphocellulose chromatography. recA protein concentration was determined by the method of Lowry et al. (14) with BSA as standard.

Purification of radiolabeled λ repressor. E. coli strain 294 containing plasmid pKB277 (15) was grown in low sulphate medium (16) containing 0.1mM $MgSO_4$ and labeled with $H_2^{35}SO_4$ (New England Nuclear) at 10 $\mu Ci/ml$ until stationary phase. The cells were resuspended in 100mM Tris-HCl (pH 8.0), 200mM KCl, 1mM EDTA, 2mM $CaCl_2$, 10mM $MgCl_2$, 1mM dithiothreitol, 5% glycerol, were sonicated and debris removed by centrifugation. Following $(NH_4)_2SO_4$ precipitation the protein was purified by chromatography on carboxymethyl cellulose and DEAE-cellulose. Repressor concentration was determined spectrophotometrically, assuming 1 mg/ml yields Abs₂₈₀ of 1.18 (17). Repressor was stored at -80°C in the presence of 100 $\mu g/ml$ inactivated BSA.

Preparation of DNAs. Colicin E1 plasmid was purified from strain JC411 thy⁻ (18) by the cleared lysate and dye-buoyant density technique (18). Supercoiled plasmid was linearized by digestion with restriction endonuclease EcoRI generously provided by John Rosenberg and Pat Greene. DNA was denatured in a boiling water bath for 5 min followed by quick chilling in ice water. λ DNA and [³²P]-labeled bacteriophage λ imm⁴³⁴ DNA were prepared as previously described (19).

pgroEL and labeled restriction fragment. The 7000 base pair plasmid pgroEL and a 5' [³²P]-labeled 350 base pair HinfI-SmaI restriction fragment were the generous gifts of Carol Woolford and Roger Hendrix.

Other Reagents. BSA was dissolved in 10mM Tris-HCl, (pH 8.0), 1mM EDTA, passed through a sterile 0.22 μm filter and inactivated by heating at 68° for 3 hrs. ATP γ S was purchased from Boehringer Manneheim. ATP, dATP, and ADP were acquired from Sigma. SSB was a gift from J.W. Chase.

λ repressor cleavage assay. λ repressor cleavage assays (30 μl) contain-

ed 20mM Tris-HCl (pH 8.0), 2mM KPO₄, 0.1mM EDTA, 5% glycerol, 30 µg/ml purified recA protein, 2 µg/ml [³⁵S]labeled λ repressor [5 x 10⁴ cpm/µg] and the indicated quantities of divalent cation, DNA, and nucleoside triphosphate. Unless otherwise indicated, reactions were incubated at 37°C for 1 hr and terminated by addition of one-third volume 0.25M Tris-HCl (pH 6.8), 3% (w/v) SDS, 20% (v/v) 2-mercaptoethanol, 0.05% bromophenol blue, 20% glycerol and immersed in a boiling water bath for 2 min. Samples were analyzed by electrophoresis on 15% SDS-polyacrylamide gels and subsequent autoradiography. Cleavage was quantitated by densitometry and integration of the peak corresponding to fragment R1 compared to a sample of thoroughly cleaved repressor (11).

ATPase activity. ATPase activity was determined in 20 µl mixtures containing 50mM Tris-HCl (pH 8.0), 0.5mM EDTA, 0.5mM 2-mercaptoethanol, 5% glycerol, 100 µg/ml inactivated BSA, 1mM [2,8-³H]-ATP (1mCi/mmol, New England Nuclear), 1.1 µg recA protein, and the indicated amount of divalent cation and DNA. Incubations were carried out at 37°C for 30 minutes after which 2 µl of reaction mixture was spotted on Brinkmann MN 300 polyethyleneimine thin layer chromatography sheets. Samples were spotted over with unlabeled ADP and chromatographed with a front of 0.5M LiCl and 1M formic acid. ADP spots were located with short wave UV light, cut out of the thin layer sheet, and counted using NEN-963 scintillation fluid.

RecA catalyzed renaturation of DNA. The ability of recA to renature homologous single stranded DNA (1) was determined in 30 µl reactions containing 20mM Tris (pH 8.0), 0.1mM EDTA, 5% glycerol, 100 µg/ml inactivated BSA, 1mM ATP, 1.1 µg recA protein, 500 ng denatured [³²P]labeled λimm⁴³⁴ DNA, and the indicated divalent cations. After 30 minute incubation at 37°, 3 µl of 50% glycerol, 0.1% bromophenol blue, 0.1% xylene cyanole FF was added and the samples immediately analyzed by electrophoresis on 0.7% agarose gels and autoradiography. We found it necessary to use radioactive DNA for these assays as cobalt quenched the fluorescence of DNA-ethidium bromide complexes.

DNA binding assays. Binding of recA protein to ssDNA was performed in 100 µl volumes containing 20mM Tris-HCl (pH 8.0), 0.1mM EDTA, 4mM MgCl₂, 20mM KCl and 100 µg/ml BSA. The ssDNA employed was [³H]-labeled viral fd DNA (Miles Biochemicals) [3x10⁴ cpm/µg] at a concentration of 0.1 µg/ml. After 5 min or longer incubation at room temperature, the mixtures were filtered through Millipore membranes (HAWP) previously treated with 0.5 M KOH as described (20). The filters were washed with 2 ml of binding buffer without BSA, and counted.

Dissociation of recA from ssDNA-NTP complexes. The determinations of

recA protein dissociation from ssDNA were performed as described by McEntee et al. (21). Reaction volumes of 1 ml contained binding buffer (20mM Tris-HCl (pH 8), 0.1mM EDTA, 4mM MgCl₂, 20mM KCl and 100 µg/ml BSA), 160 ng [³H]-fd DNA [3x10⁴ cpm/µg] and 3.3 µg of recA protein. The reaction was allowed to equilibrate for 20 min at room temperature in the presence or absence of 1mM CoCl₂. Two 100 µl aliquots were filtered through KOH-treated Millipore filters before adding the nucleoside triphosphate (1mM) together with excess unlabeled fd DNA (5 µg). Samples were removed at different times and filtered. The filters were washed with 3 ml of binding buffer without BSA and dissolved in scintillation fluid. Reducing agents were avoided in these determinations.

D-loop formation detected by membrane filtration (20). 350 µl reactions contained 20mM Tris HCl (pH 8.0), 10mM MgCl₂, 20mM KCl, 0.1mM EDTA, 15 µg/ml inactivated BSA, 1mM ATP, 12 µg/ml SSB, 1.8 µg/ml [³H] - ColEI supercoiled DNA (25% relaxed), denatured ssDNA fragments and recA protein as indicated. Incubation was at 37°C. 45 µl aliquots were mixed with 10 µl 5% Sarkosyl N1-30, and incubated on ice 3-5 min, diluted with 1 ml of ice cold 1.5 M NaCl, 0.15 M Na citrate, and filtered through Millipore HAWP membranes previously soaked in assay buffer. The filters were washed with 1 ml of 1.5 M NaCl, 0.15 M Na citrate and counted.

D-loop formation by agarose gel electrophoresis. 20 µl reactions contained 30mM Tris-HCl (pH 8.0), 10mM MgCl₂, 1mM KPO₄, 2mM ATP, 100 µg/ml inactivated BSA, 1 µg/ml pgroEL plasmid DNA, 0.1 µg/ml denatured 5'-[³²P] - HinfI/SmaI restriction fragment, and recA protein. After incubation at 37° for 4 min, 4 µl of 0.1 M EDTA (pH 8.0), 2% sarkosyl, 0.05% xylene cyanole FF, 0.05% bromophenol blue, 25% glycerol was added, the mixtures incubated at 17°, and analyzed on 0.6% agarose gels.

RESULTS

CoCl₂ increases the proteolytic activity of recA protein. When different divalent cations were tested in the recA dependent cleavage assay of λ repressor performed with ssDNA and dATP, we found that Mn²⁺ and Zn²⁺ were ineffective, and that Mg²⁺, Ca²⁺ or Co²⁺ could fulfill the requirement of the reaction (Fig. 1). On a molar basis, the most active cation was Co²⁺, followed by Ca²⁺ or Mg²⁺.

Addition of CoCl₂ to the standard reaction containing MgCl₂ or CaCl₂ enhanced the rate of repressor cleavage (Fig. 2). The optimal concentration of CoCl₂ was found to be 1mM in the presence of 4mM MgCl₂. Similar results were obtained with tif protein promoted cleavage. The rates of repressor cleavage

Figure 1. recA directed cleavage of λ repressor with various divalent cations. λ repressor cleavage reactions were performed as in the Materials and Methods with 1mM dATP, 1.5 μ g/ml denatured ColE1 plasmid DNA, and the given concentrations of the indicated divalent cation. Samples were analyzed on a SDS-polyacrylamide gel and autoradiography. R, R₁, and R₂ refer to λ repressor, and repressor fragments R₁ and R₂ respectively.

sustained by MgCl₂, CaCl₂ and CoCl₂, with 5mM ATP are shown in Fig. 3. The rate attained with cobalt was 2 times faster than that obtained with magnesium alone.

Influence of CoCl₂ in the repressor cleavage performed with different nucleoside triphosphates. The only nucleoside triphosphates that permit signi-

Figure 2. Stimulation of cleavage by addition of CoCl₂. Cleavage reactions were prepared with 1mM dATP, 1.5 μ g/ml denatured ColE1 plasmid DNA, the indicated amounts of CoCl₂, and 4mM MgCl₂ (○) or 4mM CaCl₂ (●). The percent repressor cleaved was determined as described in the Materials and Methods.

Figure 3. Rate of λ repressor cleavage in the presence of various divalent cations. Cleavage reactions were prepared as described under Materials and Methods with 5mM ATP, 1.5 μ g/ml denatured ColE1 plasmid DNA, and 4mM MgCl₂ (●), 4mM MgCl₂ and 1mM CaCl₂ (○) or 4mM MgCl₂ and 1mM CoCl₂ (■). At the indicated times 30 μ l aliquots were withdrawn from each reaction and analyzed as described in the Materials and Methods.

ficant levels of repressor cleavage are ATP, dATP and ATP_γS (reference 11 and Table I). ATP is the least effective because it undergoes hydrolysis to ADP, which is an inhibitor of the reaction. Addition of 0.5mM ADP to reactions containing 1mM ATP_γS significantly reduced the rate of cleavage (data not shown). Table I shows that the presence of 1mM CoCl₂ increased the rate of cleavage with all three nucleoside triphosphates. With 1mM ATP, the increase in rate was 16 fold; with dATP it was 1.7 fold, while in the presence of ATP_γS, which supports the highest rate in the absence of cobalt, it was enhanced only 1.2 fold.

CoCl₂ does not alter recA ATPase activity. ATP_γS is an effective nucleoside triphosphate in the proteolytic cleavage of repressor (reference 10 and Table I). Since it is not efficiently hydrolyzed (22), nucleoside triphosphatase activity must not be required for proteolysis. Given the fact that CoCl₂ increased recA proteolytic activity with ATP, it was important to determine if the stimulation was due to a diminished ATPase activity, thereby preventing accumulation of ADP. First, it was observed that CoCl₂ alone did not replace the requirement for Mg²⁺ (2,4) in the ssDNA-dependent hydrolysis of ATP by recA protein. The optimal concentration of MgCl₂ remained the same (4mM) in the presence of 1mM CoCl₂ (data not shown). Fig. 4 shows that ad-

TABLE I. ENHANCEMENT OF CLEAVAGE BY CoCl₂ WITH VARIOUS NUCLEOSIDE TRIPHOSPHATES.

NTP	CoCl ₂	% Repressor Cleared	$\frac{\% \text{ Cleaved} + \text{Co}}{\% \text{ Cleaved} - \text{Co}}$
—	—	0	
—	1mM	0	—
ATP	—	2.7	
	1mM	44.2	16.4
dATP	—	35.8	
	1mM	62.5	1.74
ATP γ S	—	47.7	
	1mM	56.0	1.17

Repressor cleavage assays were performed as described under Materials and Methods with 4mM MgCl₂, 1.5 μ g/ml denatured ColE1 plasmid DNA, 1mM of the indicated nucleoside triphosphate, and CoCl₂ as indicated.

dition of 1mM CoCl₂ did not significantly alter either the DNA dependency of the ATPase activity nor the rate of the reaction. Concentrations of CoCl₂ up to 3mM were not significantly inhibitory (Fig. 4, inset). When concentrations of CoCl₂ higher than 1mM were used, we eliminated or lowered the concentration of β -mercaptoethanol or dithiothreitol to prevent precipitate formation.

These experiments indicate that the increased proteolytic activity of recA protein with CoCl₂ is not due to a change in the ATPase activity.

Co²⁺ stimulates D-loop formation. To determine if CoCl₂ affected the formation of D-loops by recA protein, 2mM CoCl₂ was added to D-loop assays containing 10mM MgCl₂, 12 μ g/ml SSB, and 34 μ g/ml recA protein. In the presence of CoCl₂, the rate and extent of D-loop formation was increased (Fig. 5). Furthermore, the dissociation of D-loops in the presence of excess recA protein (23) was also reproduced in our control experiments which contained SSB but was not observed when CoCl₂ was present. CoCl₂ also enhanced D-loop activity at limiting recA concentrations (data not shown).

The stimulation of strand assimilation in the presence of CoCl₂ was further demonstrated by an agarose gel electrophoresis assay for D-loops. In this

Figure 4. Effect of CoCl₂ on *recA* mediated ATPase. The *recA* protein ATPase activity was determined as described under Materials and Methods with the indicated amounts of denatured ColE1 plasmid DNA, 4mM MgCl₂ (○) or 4mM MgCl₂ and 1mM CoCl₂ (●). Each point represent the average of two independent determinations. INSET ATPase reactions contained 9 μg/ml denatured ColE1 plasmid DNA, 4mM MgCl₂, and the CoCl₂ as indicated. The 2-mercaptoethanol was omitted from these reactions to prevent precipitate formation at higher CoCl₂ concentrations.

Figure 5. Membrane filtration assay of D-loop formation. 34 μg/ml *recA* protein was added to D-loop reactions containing 2mM CoCl₂ (closed symbols) or no CoCl₂ (open symbols), an 1.7 μg/ml denatured ColE1/HinfI fragments (●,○) or λ/HinfI fragments (■,□). After incubation at 37° for various times, 45 μl aliquots were withdrawn and filtered as described in the Materials and Methods.

assay, a 5'-[^{32}P]- restriction fragment (350 bases) of plasmid pgroEL was reacted with intact plasmid. Strand assimilation is demonstrated by the presence of radioactivity at the position of full length plasmid on agarose gels. Addition of 2mM CoCl_2 to reactions containing 10mM MgCl_2 and increasing amounts of recA protein allowed the detection of heteroduplexes over a range of recA concentrations (Fig. 6). In all assays, strand assimilation required ATP and could not be detected with heterologous ColE1 plasmid DNA (data not shown).

CoCl_2 inhibits the recA catalyzed DNA renaturation. In an effort to explore the mechanism of CoCl_2 enhanced proteolytic activity, we studied the ability of recA protein to catalyze ssDNA reannealing. The reactions were performed as described by Weinstock et al. (1). [^{32}P]-labeled denatured λ DNA was used because the presence of CoCl_2 quenched the normal fluorescence of DNA complexed with ethidium bromide. Figure 7 is an autoradiogram of denatured [^{32}P]-labeled λ imm⁴³⁴ DNA analyzed by gel electrophoresis after exposure to

Figure 6. Agarose gel electrophoresis assay for D-loop formation. recA protein was added to reaction mixtures prepared as described in the Materials and Methods containing no CoCl_2 (lanes 1-6) or 2mM CoCl_2 (lanes 7-12). After 4 min incubation at 37° the samples were deproteinized and electrophoresed. Lanes 1 and 7, no recA protein. Lanes 2 and 8, 2.8 $\mu\text{g/ml}$ recA, lanes 3 and 9, 5.5 $\mu\text{g/ml}$ recA, lanes 4 and 10, 8.3 $\mu\text{g/ml}$ recA, lanes 5 and 11, 11 $\mu\text{g/ml}$ recA, lanes 6 and 12, 13.7 $\mu\text{g/ml}$ recA. Lane 13, native fragment DNA marker.

Figure 7. recA catalyzed renaturation of DNA in the presence of various divalent cations. The ability of recA to renature denatured [³²P] labeled λ imm⁴³⁴ DNA was assayed as described under Materials and Methods. Reactions contained 1.1 μ g of recA protein where indicated and the given millimolar concentrations of divalent cation. Channel 1 contains a sample of the original denatured DNA.

recA protein in the presence of different divalent cations and ATP. Without recA, the DNA remained low molecular weight (channel 2). In the presence of MgCl₂, recA promoted the increase in molecular weight of DNA and at 8mM MgCl₂ the majority of the labeled DNA did not enter the gel due to renaturation (channel 11-13). CaCl₂ alone did not promote this increase (channels 7-10), and did not interfere with the reannealing effected by MgCl₂ (channels 18-20). Addition of CoCl₂ differed from CaCl₂ in the fact that even at 2mM, it inhibited the DNA aggregation usually obtained with 8mM MgCl₂ (channels 15-17). It can be concluded that the presence of CoCl₂ blocks the recA-catalyzed DNA renaturation reaction.

Affinity of recA protein for DNA is affected by CoCl₂. As shown above, cobalt prevented the Mg dependent ssDNA renaturation reaction of recA. In

Figure 8. *recA* binding to ssDNA. The ability of *recA* protein to retain [^3H]-labeled fd DNA on Millipore filters was measured in the presence of 4mM MgCl_2 (○) or 4mM MgCl_2 and 1mM CoCl_2 (●) as described under Materials and Methods. Binding reactions were supplemented with no nucleoside triphosphate (a), 2mM ATP (b), 1mM $\text{ATP}\gamma\text{S}$ (c), or 1mM dATP (d). Each point represents the average of two determinations.

this regard, the effect of cobalt was similar to $\text{ATP}\gamma\text{S}$ (1) and suggested that cobalt might alter the affinity of *recA* for DNA or its release, even though ATPase activity was permitted. This was found to be the case in ssDNA binding assays (Fig. 8). The assays were performed with [^3H]-labeled viral fd DNA, to avoid the renaturation affected by *recA* protein under these conditions. We measured the DNA-*recA* complex retained on Millipore filters after equilibrium was reached at room temperature. Reaction mixtures were prepared with each one of the nucleoside triphosphates in the presence and absence of 1mM CoCl_2 . Fig. 8 contains the results of several experiments which establish that in the presence of MgCl_2 , CoCl_2 increased the affinity of *recA* for ssDNA with all three nucleoside triphosphates. Shibata et al. (24) have attributed the increased stability of *recA*-DNA complexes when $\text{ATP}\gamma\text{S}$ is present as cofactor to an enhanced binding affinity of *recA*, due to the fact that $\text{ATP}\gamma\text{S}$ is not hydrolyzed. It has also been speculated that hydrolysis of NTP is involved in the release of *recA* protein from the DNA complex (5). The interesting fact, in the case of CoCl_2 is that hydrolysis of ATP is not

Figure 9. Dissociation kinetics of *recA* protein-ssDNA complexes. 3.3 μ g of *recA* protein were complexed to 160 ng [³H]-fd DNA [3×10^4 cpm/ μ g] in 1 ml of binding buffer with 1mM CoCl₂ (○) or without CoCl₂ (●) for 20 min at room temperature. After removing two aliquots for 0 time determinations, the nucleoside triphosphate (1mM) and excess cold fd DNA (5 μ g) were added simultaneously. 100 μ l aliquots were filtered at the indicated times.

inhibited (see above), nevertheless there is increased binding with ATP (Fig. 8). For this reason we investigated the dissociation of *recA* protein from ssDNA complexes. We found that addition of CoCl₂ increased the half life of *recA*-ssDNA complexes with the three nucleotide triphosphates tested (Fig. 9). No change was observed without nucleoside triphosphate, with an estimated half-time of the complexes under these conditions of 10-12 min. (Fig. 9). As reported by McEntee et al. (21), the addition of ATP releases the DNA in a matter of seconds, but CoCl₂ stabilized the complexes slowing down the dissociation to about 2 min half-time. dATP also caused rapid dissociation of the order of 1 min half-time, which decreased to 3 min in the presence of CoCl₂. Even when ATPγS was added to the *recA*-ssDNA complex, stabilizing the binding to the point where DNA is released at more than 60 min half-time (21) CoCl₂ increased its stability (Fig. 9).

Since the association kinetics were too fast to be measured, we conclude that a large part of the increased affinity observed with CoCl₂ can be attributed to an increased stability of the *recA*-ssDNA-NTP complexes promoted by this metal.

DISCUSSION

We have found that CoCl_2 increased the rate of recA-catalyzed endoproteolytic cleavage of λ repressor, when compared to reactions containing MgCl_2 or CaCl_2 , and enhanced the rate of cleavage when used in conjunction with these cations. To explore which parameters of the reaction were modified by cobalt, we investigated the effect of CoCl_2 on the ATPase activity of this enzyme. The rationale being that λ repressor cleavage can proceed without hydrolysis of ATP (i.e., with $\text{ATP}_{\gamma}\text{S}$) (11) and is inhibited by accumulation of ADP. Therefore, cobalt could enhance the reaction by inhibiting the ATPase activity of recA protein. We found that this was not the case, since the presence of CoCl_2 did not affect recA-mediated hydrolysis of ATP (Fig. 2). Another parameter of importance in the proteolytic reaction is the affinity of the participating proteins for ssDNA. We observed that recA protein had increased binding to ssDNA in the presence of CoCl_2 when ATP, dATP or $\text{ATP}_{\gamma}\text{S}$ were used as cofactors (Fig. 8). In addition, the dissociation of recA protein-ssDNA-NTP complex was delayed by CoCl_2 (Fig. 9). These results suggest that the increase in proteolytic activity may be due to the stable association of recA protein with ssDNA. Whether cobalt modifies the protein or the DNA cannot be ascertained at present. The observation that DNA intercalated with ethidium bromide loses its fluorescence when CoCl_2 is present may be an indication of a conformational change in DNA. The fact that cobalt can induce modification of another enzyme, terminal deoxynucleotidyl transferase, which requires ssDNA as a primer for the addition of NMP to the 3'OH end with Mg^{2+} as cofactor, but will accept duplex DNA when Co^{2+} is used instead (25) may indicate a more general tendency of this metal to affect protein-DNA interactions. Preliminary experiments with λ repressor have shown that 1mM CoCl_2 also affects this protein binding to DNA (unpublished data). We do not know whether cobalt is an effector *in vivo*. Attempts at inducing *E. coli* lysogens by the addition of CoCl_2 to the cultures have been unsuccessful.

We found that other enzymatic activities of recA protein were also modified by Co^{2+} , namely the ssDNA renaturation reaction and the formation of D-loops. Under conditions where the enzyme promotes reannealing of ssDNA (1) the addition of 2mM CoCl_2 or higher concentrations inhibited the reaction, while CaCl_2 had no effect (Fig. 7). $\text{ATP}_{\gamma}\text{S}$ is also known to inhibit the recA-directed reannealing reaction (1), although the mechanism is not known. $\text{ATP}_{\gamma}\text{S}$ inhibits the recA ATPase activity (24) and retards dissociation of recA from ssDNA complexes (21), suggesting that either of these activities may be necessary for reannealing. The observation that CoCl_2 does not significantly alter

the recA ATPase activity but does prevent strand reannealing proves that ATP hydrolysis is not the only requirement for reannealing.

The formation of D-loops was found to be enhanced by the presence of cobalt. This metal seems to increase the rate of strand assimilation and also inhibits the dissociation observed at high recA protein concentrations (Fig. 5). The convenience of using cobalt for D-loop formation was even more evident when a small ssDNA fragment (350 bases) was complexed with the homologous duplex DNA under conditions which usually requires SSB to be detectable (Fig. 6). We did not observe D-loop formation with heterologous DNA.

Superficially, it may seem contradictory that cobalt inhibits the homologous pairing of complementary strands of DNA while it enhances the D-loop formation, catalyzed by the same enzyme. Analysis of the factors required by these reactions reveal that they are indeed different. Reannealing of denatured DNA is accomplished with catalytic amounts of recA protein and requires hydrolysis of ATP, while the D-loop reaction is carried out with stoichiometric quantities of recA protein and can proceed without hydrolysis of ATP (26). Our finding that cobalt decreases the dissociation rate of recA protein from ssDNA in the presence of ATP suggests that for the reannealing reaction the fast dissociation of recA protein is essential, while the D-loop formation benefits from a long-lasting association of this protein with DNA. The action of cobalt reinforces the similarities already established between D-loop formation and repressor cleavage.

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ABBREVIATIONS

ATP_γS, adenosine-5'0(3-thiotriphosphate); ssDNA, single strand DNA; BSA, bovine serum albumin; SSB, single strand binding protein.

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