RecA Protein-promoted Cleavage of LexA Repressor in the Presence of ADP and Structural Analogues of Inorganic Phosphate, the Fluoride Complexes of Aluminum and Beryllium*

(Received for publication, June 20, 1988)

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Complexes formed from Al\textsuperscript{3+} or Be\textsuperscript{2+} and fluoride inhibit the single-stranded DNA-dependent ATPase activity of RecA protein. In contrast, poly(dT)-RecA-ADP complexes, which are inactive for cleavage of LexA protein, become fully active in the presence of AlF\textsubscript{3} or BeF\textsubscript{2} ions. These data suggest that fluoride complexes of aluminum and beryllium (called herein X) convert RecA-ADP complexes, which bind weakly to single-stranded DNA, into RecA-ADP-X complexes, which bind tightly to single-stranded DNA, the ADP-X moiety behaving as a nonhydrolyzable analogue of ATP. We propose that AlF\textsubscript{3} and BeF\textsubscript{2} ions act as analogues of inorganic phosphate by binding to the site of the \(\gamma\)-phosphate of ATP on RecA-ADP complexes, hence mimicking the single-stranded DNA-RecA-ADP-P, transition state. We conclude that the elementary reaction that switches RecA protein from a high affinity single-stranded DNA binding state to a low affinity single-stranded DNA binding state is not ATP hydrolysis per se but P\textsubscript{i} release.

The RecA protein of Escherichia coli has been shown to play an essential role in genetic recombination and in the induction of at least 17 genes which are negatively controlled by the LexA protein (Walker, 1985). The latter activity of the RecA protein results from its capacity to promote cleavage of the LexA repressor (Slilaty and Little, 1987). RecA-promoted proteolysis is highly specific because, besides the LexA protein, the RecA protein promotes the cleavage of one other bacterial protein, UmuD (Burckhardt et al., 1988), and that of bacteriophage repressors, such as the \(\lambda\) of repressor (Roberts and Roberts, 1981); all these proteins share structural homologies (Nohmi et al., 1988). RecA-promoted proteolysis is not constitutively expressed in bacteria; RecA protein must be activated in order to mediate cleavage of proteins (Moreau et al., 1980a; Quillardet et al., 1982). The activation of RecA protein occurs when DNA replication is perturbed for example by the presence of DNA lesions generated by carcinogenic agents (Moreau et al., 1980b). However, it is still unclear exactly what constitutes the inducing signal for the activation of the RecA protein.

The biochemical evidence suggests that RecA protein is activated to cleave repressors when it forms a ternary complex with a polynucleotide, such as poly(dT), and a nucleoside triphosphate, such as ATP or dATP (Craig and Roberts, 1980; Phizicky and Roberts, 1981; Cotterill et al., 1982). It is generally thought that the requirement in vitro of a polynucleotide for proteolysis reflects in vivo the generation of ssDNA regions by inducing agents. The role of the second cofactor required for the activation of the RecA protein, the nucleoside triphosphate, is less clear. RecA protein exhibits a polynucleotide-dependent ATPase activity (Roberts et al., 1979). However, binding of ATP but not its hydrolysis is required for proteolysis since ATP-\(\gamma\)-S, a nonhydrolyzable analogue of ATP, can substitute for ATP in RecA-dependent proteolytic processes (Craig and Roberts, 1981; Slilaty and Little, 1987). The hydrolysis of ATP to ADP and inorganic phosphate is, however, intricately coupled to the activity of RecA protein since ADP inhibits RecA-dependent processes (McEntee et al., 1981; Cox et al., 1983; Menetski and Kowalczykowski, 1987). It has been suggested, therefore, that RecA protein exists in two different states: a high affinity ssDNA binding state when ATP is bound to RecA protein, and a low affinity ssDNA binding state when ADP is bound to RecA protein (Menetski et al., 1988).

It is an important issue to understand how the elementary reactions of ATP hydrolysis, including the release of P\textsubscript{i}, regulate the activities of the RecA protein by switching it from a high to a low affinity ssDNA binding state. In order to investigate these processes, we studied the effects of structural analogues of phosphate, the fluoride complexes of aluminum and beryllium, on two RecA-dependent reactions, namely ATP hydrolysis and cleavage of LexA protein. It has been proposed that AlF\textsubscript{3} and BeF\textsubscript{2} complex ions, which have the same tetrahedral geometry and bond lengths as inorganic phosphate, act by mimicking the \(\gamma\)-phosphate of ATP or GTP, thereby converting various enzymes from a conformation normally observed with GDP or ADP to a conformation observed with nucleoside triphosphates (Sternweis and Gilman, 1982; Bigay et al., 1985; Lange et al., 1986; Robinson et al., 1986; Bigay et al., 1987; Carlier et al., 1988). These analogues may therefore be useful to probe the catalytic mechanism of nucleotidases involved in energy transduction.

MATERIALS AND METHODS

RecA and LexA proteins were purified as previously described (Moreau, 1987). RecA protein was stored in 20 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, 1 mM dithiorthreitol, and 10% (w/v) glycerol at -80°C; its concentration was determined assuming an A\textsubscript{280} of 5.9. LexA protein was stored in 10 mM Pipes, pH 7.0, 0.1 mM EDTA, 1 mM dithiorthreitol, 200 mM NaCl, and 10% (w/v) glycerol at -80°C; its concentration was determined assuming an A\textsubscript{280} of 3.2. Poly(dT) was obtained from Pharmacia LKB Biotecnology Inc.; it was assumed that a solution of 50 \(\mu\)g/ml has an absorbance at 260 nm of 1.0. ATP

* This work was supported in part by Grant BI16-145-F from Euratom, Association pour la Recherche sur le Cancer, Ligue Nationale contre le Cancer, and Fondation pour la Recherche Medicale. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: ssDNA, single-stranded DNA; Pipes, 1,4-piperazinediethanesulfonic acid.
RESULTS

Inhibition of RecA Protein ATPase Activity by Fluoride Complexes of Aluminum and Beryllium—It has been previously shown that in the presence of 1-10 mM fluoride, AIF
3 ions at micromolar concentrations inhibit the glucose-6-phosphatase (Lange et al., 1986) and the (Na + K)-ATPase (Robinson et al., 1986). We found that in the presence of 10 mM NaF, 17 

\mu M aluminum nitrate strongly inhibited the ATPase activity of the RecA protein (Table I). NaF alone weakly inhibited RecA protein activity, whereas aluminum nitrate was totally ineffective in the absence of NaF. The inhibiting effect of NaF was not unexpected. Although plastic tubes and pipetting devices were used at all times to prevent etching by fluoride of aluminum from glassware, it is known that aluminum fluorosilicates are common impurities in commercial preparations of NaF and ATP (Sternweis and Gilman, 1982).

By using a reaction mixture containing 1 \mu M RecA protein and 2.5 mM ATP, the rate of ATP hydrolysis was linear for 90 min, at which time approximately 50% ATP was hydrolyzed to ADP and P\(_i\) (Fig. 1). The rate of ATP hydrolysis then decreased rapidly as a consequence, probably, of the accumulation of ADP (Cox et al., 1983). In the presence of 10 mM NaF and 10 \mu M aluminum nitrate, the hydrolysis of ATP proceeded at a normal rate for 10 min and then was gradually inhibited with time; the rate of ATP hydrolysis was 90% inhibited after 60 min of incubation. The addition of 50 \mu M aluminum nitrate produced a complete inhibition after 10 min of incubation, while approximately 100 \mu M ATP (4% of the input concentration) was hydrolyzed. These results indicate that fluoride complexes of aluminum, probably as AlF\(_3\) (Lange et al., 1986), prevent the turnover of ATP on RecA protein. The nonlinear kinetics of ATP hydrolysis observed in the presence of AlF\(_3\) are consistent with a slow binding of the fluoride complexes to the RecA protein as compared to the other reactions involved in the hydrolysis of ATP.

In the case of activation of GTP-binding proteins, it has been shown that among various metal ions other than Al\(^{3+}\), only Be\(^{2+}\) can form active complexes with fluoride, mainly as BeF\(_3\) (Sternweis and Gilman, 1982; Bigay et al., 1987). Indeed, we found that Be\(^{2+}\) ions inhibit RecA protein ATPase activity in the presence of NaF (Fig. 2), but the extent of inhibition observed over the range of concentrations of beryllium sulfate tested (0-100 \mu M) indicates that BeF\(_3\) binds to RecA protein more weakly than does AlF\(_3\).

AlF\(_3\) Inhibits ATP Hydrolysis but Stimulates LexA Protein Cleavage—Does AlF\(_3\) inhibit all RecA protein activities like ADP, which decreases the binding affinity of RecA protein for polynucleotides? We can answer this question by testing for another activity of the RecA protein which can be expressed in the absence of ATP hydrolysis but which nevertheless requires the formation of a stable complex of RecA protein with ssDNA, i.e. cleavage of LexA protein (Silliaty and Little, 1987).

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**TABLE I**

Inhibition of RecA protein ATPase activity by AlF\(_3\)

| Additions to assay mixture | None | NaP\(_3\) | Al\(^{3+}\) | NaF | NaF + Al\(^{3+}\) |
|---------------------------|------|----------|-----------|-----|-----------------|
| % ATP hydrolyzed\(^a\)    | 43   | 44       | 43        | 31  | 7 ± 0.5\(^c\)  |
| % inhibition              | 0    | 0        | 28        | 84  |                 |

\(^a\) Addition to low salt medium of NaP\(_3\) (10 mM), or NaF (10 mM), and/or Al(NO\(_3\))\(_3\) (17 \mu M).

\(^c\) Incubation for 90 min.

\(^c\) Mean of three determinations.
Activation of ssDNA-RecA-ADP by AlF₃

Cleavage conditions differed from the standard ATPase assay conditions that were used for the kinetic measurements described above essentially in that salt concentration was increased in order to reduce the rate of LexA cleavage. As seen in Fig. 3, in the absence of aluminum fluoride, ATP hydrolysis and LexA cleavage proceeded respectively to 34% and approximately 50% completion in 15 min; both reactions were then essentially inhibited probably as a consequence of the accumulation of ADP (Fig. 3). In contrast, in the presence of AlF₃, whereas ATP hydrolysis was rapidly inhibited, cleavage of LexA protein could proceed to completion within 45 min of incubation. We can therefore conclude that in the presence of AlF₃ and ATP, RecA protein binds to poly(dT) and is activated to promote LexA protein cleavage although it no longer hydrolyzes ATP.

These results were not indicative of the nature of the nucleotide bound to the RecA molecules which promoted LexA cleavage. The nucleotide site on these RecA molecules could be either occupied by AlF₃ that could induce a ATP-like conformation of the protein, or it could be occupied by ATP whereas AlF₃ would inhibit ATP hydrolysis through its binding to another site, or it could be occupied by a ADP-AlF₃ complex that would behave as a nonhydrolyzable analogue of ATP. In order to test these different hypotheses, the cleavage of LexA protein was assayed in the presence of AlF₃ or ADP, or both.

Poly(dT)-RecA-ADP Complexes Cleave the LexA Protein in the Presence of AlF₃—No cleavage of LexA protein occurred in the presence of RecA protein and poly(dT) upon the addition either of ADP (Fig. 4) or of AlF₃ (10 mM NaF plus 10 mM Al(NO₃)₃) (data not shown). Although dADP reduces the apparent affinity of X RecA protein for ssDNA less than ADP (Menetski et al., 1988), no cleavage was observed when dADP substituted for ADP (Fig. 4). In contrast, cleavage of the LexA protein reached nearly 100% completion within 60 min of incubation in the presence of both ADP and AlF₃ as well as in the presence of ATP and AlF₃. Cleavage was slightly more efficient in the presence of dADP and AlF₃ than in the presence of ADP and AlF₃, a result that is in good agreement with the hypothesis that dADP binds more tightly to RecA protein than does ADP (Kowalczykowski, 1986). No cleavage of LexA protein occurred, however, in the presence of AlF₃ and dADP when poly(dT) was omitted from the reaction mixture (Fig. 5), indicating that AlF₃ does not release RecA protein from the requirement of a polynucleotide to be active. Taken together, these results indicate that ssDNA-RecA-ADP-AlF₃ complexes are as active as ssDNA-RecA-ATP complexes for cleavage of the LexA protein. The final extent of the reaction could be even higher in the presence of either ADP or ATP and AlF₃ than in the presence of ATP alone since AlF₃ prevents the inhibition due to the accumulation of ADP.

BeF₅ Activates RecA-dADP Complexes Less Efficiently Than Does AlF₃—In a reaction mixture containing 1 µM RecA
tion mixtures contained either increasing concentrations of aluminum nitrate (0–10 μM) when the concentration of NaF was held constant at 10 mM, or decreasing concentrations of NaF (10–0.25 mM) while the concentration of aluminum was held constant at 10 μM. The control reaction contained 10 mM NaF plus 10 μM aluminum nitrate but no poly(dT). Incubation was for 60 min. In the first and last lanes of the gel were run partially autodigested LexA protein. The gel was stained with Coomassie R-250. The positions of RecA, intact LexA, and COOH-terminal (Lex-C) and NH2-terminal (Lex-N) cleavage fragments of LexA protein are indicated.

![Image](image.png)

**Fig. 5. Activation of LexA protein cleavage by AlF₃.** Reaction mixtures contained either increasing concentrations of aluminum nitrate (0–10 μM) when the concentration of NaF was held constant at 10 μM. The control reaction (Ct) contained 10 mM NaF plus 10 μM aluminum nitrate but no poly(dT). Incubation was for 60 min. In the first and last lanes of the gel were run partially autodigested LexA protein. The gel was stained with Coomassie R-250. The positions of RecA, intact LexA, and COOH-terminal (Lex-C) and NH2-terminal (Lex-N) cleavage fragments of LexA protein are indicated.

![Image](image.png)

**Fig. 6. Activation of LexA protein cleavage by AlF₃, BeF₃ or PO₄.** Reaction mixtures contained 10 mM NaF and increasing concentrations of aluminum nitrate (0–80 μM) (Al) or beryllium sulfate (0–1.6 mM) (Be), or increasing concentrations of NaF (0–0.1 M) (P). Incubation was for 60 min. The first lane of the gel contained partially autodigested LexA protein. The gel was stained with Coomassie R-250.

![Image](image.png)

**Fig. 7. Tentative scheme featuring the elementary steps in ssDNA-dependent ATP hydrolysis by RecA protein, and the conformational changes of RecA protein linked to P, release.** In this model, RecA protein can be either free or bound to ssDNA (---). Rectangles represent the conformation which binds ssDNA tightly, and triangles the conformation which binds ssDNA weakly. RecA-ATP binds more tightly to ssDNA than RecA-ADP, that is the equilibrium association constant Lₜ is larger than Lₕ. ATP hydrolysis occurs via the formation of a transient ssDNA-RecA-ADP-Pi complex (X is then equivalent to P), P, release is linked to the destabilization of the ssDNA-RecA complex. Analogues of P, (AlF₃ or BeF₃ called herein X) bind to RecA-ADP with an equilibrium association constant Kₓ and to ssDNA-RecA-ADP with a corresponding association constant Kₓ. Binding of X to RecA protein shifts equilibria toward the highly stable ssDNA-RecA-ADP-X complex, in which X mimics P, in the transient ssDNA-RecA-ADP-Pi state, i.e., Lₜ > Lₕ. Since KₓLₓ = KₓLₓ X has a higher affinity for ssDNA-RecA-ADP than for RecA-ADP (Kₓ > Kₓ). The known cooperativity involved in the binding of RecA protein to ssDNA is not featured for simplicity. This cooperativity may prevent the dissociation of RecA-ADP subunits from ssDNA as long as enough ATP is available in the medium to allow a rapid exchange of ADP for ATP.
was as efficient in the presence of ATP as in the presence of ADP plus AIF₃. However, the inhibition of ATP hydrolysis by Pₐ analogues was not instantaneous. In the presence of 1 μM RecA protein, 2.5 mM ATP, and 50 μM aluminum fluoride, 125 cycles of ATP hydrolysis occurred (5% ATP was hydrolyzed) before all RecA molecules were blocked in poly(dT)-RecA-ADP-X complexes. This indicates that at high ATP concentration, the rate of binding of AIF₃ was lower than the rate of exchange of ADP for ATP. This exchange probably occurs without the release of RecA protein from ssDNA since dissociation of ssDNA-RecA complexes apparently occurs cooperatively when approximately 50% of the input ATP has been hydrolyzed (Cox et al., 1983).

These results allow us to expand the ssDNA-dependent ATPase cycle of RecA protein proposed by Menetski and Kowalczykowski (1987) (Fig. 7). Upon binding of RecA-ATP complexes to ssDNA, ATP is hydrolyzed. The hydrolysis of ATP to ADP and P_i can be divided into two distinct steps. First, cleavage of the γ-phosphate leads to the kinetic intermediate RecA-ADP-P_i, which binds tightly to ssDNA. Second, P_i release in the medium leads to the RecA-ADP complex, which binds weakly to ssDNA. Exchange of ADP for ATP might occur either in this complex or on RecA-ADP subunits following their dissociation from ssDNA; the choice between these two pathways depends on the relative concentration of ATP and ADP in the medium (Cox et al., 1983; Menetski and Kowalczykowski, 1987). Restoration of the ssDNA-RecA-ATP complex allows then a new cycle of ATP hydrolysis to occur.

According to this model, the elementary reaction that triggers a major conformation change of RecA protein leading to a large decrease in its affinity for ssDNA is not hydrolysis of ATP per se but P_i release. However, the ratio of RecA-ADP over RecA-ATP and RecA-ADP-P_i subunits that induce the dissociation of a ssDNA-RecA nucleoprotein filament is unknown at this point (Brenner et al., 1987).

RecA protein ATPase activity may represent another example of a coupled villar process (Jencks, 1980), similar to that observed with other enzymes involved in energy or signal transduction. In the case of actomyosin, P_i release is linked to the development of force (Hibberd et al., 1985); with rhodopsin, the regulation of the associated phosphodiesterase is exerted by P_i release from the complex transducin-GDP-P_i (Bigay et al., 1985; Bigay et al., 1987); with actin and tubulin, P_i release following ATP or GTP hydrolysis is also associated to a structural change causing the destabilization of actin filaments or microtubules (Carlier and Pantaloni, 1988; Carlier et al., 1988; Combeau and Carlier, 1988).

A number of factors such as ionic conditions, pH, and the presence of proteins such as Escherichia coli single-stranded DNA-binding protein are known to affect RecA protein activities, such as ATP hydrolysis, LexA protein cleavage, and strand exchange (McEntee et al., 1981; Cotterill and Fersht, 1983; Moreau and Roberts, 1984; Chow and Radding, 1985; Kowalczykowski and Krupp, 1987). It will be interesting to determine if these parameters affect the rate of P_i release and, hence, modulate RecA protein activities.

Acknowledgments—We thank D. Pantaloni for suggestions and discussions.

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