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SALT CAN FUNCTIONALLY MIMICK DNA IN ACTIVATING THE RECA PROTEIN ATPASE[1]

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ABSTRACT The recA protein of *Escherichia coli* is a DNA-dependent ATPase. Under conditions optimal for the DNA-dependent reaction, little ATP hydrolysis is observed in the absence of DNA. However, high concentrations (nearly 2 M) of a variety of salts can stimulate the ATPase to levels approximating those observed with DNA. The increase in ATP hydrolysis requires the uptake of 3 to 4 ions by recA protein. Additionally, the reaction requires self-aggregation of recA protein, and is dependent upon magnesium ions. These requirements are identical to the DNA-dependent reaction. Thus, salts appear to be functionally mimicking DNA.

INTRODUCTION

RecA protein promotes homologous recombination in *Eschericia coli* (for reviews see Refs. 1,2). This process can be reproduced *in vitro* (3). The active species in initiating DNA strand exchange is a recA/ssDNA nucleoprotein helical filament in which the DNA inside the filament is extended by 50% relative to B-form double-stranded DNA (3,4). When

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bound to DNA, recA protein hydrolyzes ATP (5). Strand exchange requires ATP hydrolysis, and the branch migration phase of the reaction proceeds in the 5' to 3' direction relative to the initially bound single-stranded DNA (ssDNA) (6,7). The mechanism by which the hydrolysis of ATP is coupled to unidirectional strand exchange is not known.

The DNA-dependent ATPase activity of recA protein has been extensively characterized (2,8). Published values for K_m, k_{cat}, K_d for DNA binding, and the Hill coefficient have to date provided little information of mechanistic significance. They exhibit a dependence variously on the concentration of ATP, recA protein, DNA (as well as its length and composition), magnesium, and other salts (8). The presence of a large multi-site ligand (DNA) in this system complicates the analysis of the ATPase activity. A simplified system which did not include DNA might be valuable in dissecting the kinetic mechanism of ATP hydrolysis.

Previous work has shown that recA protein does not apppear to interact with the nucleotide bases of DNA (9). It was suggested that recA protein binds DNA through the phosphate backbone (9). Binding to ssDNA exhibits substantial cooperativity (10,11). Single-stranded oligonucleotides with less than 50 nucleotides are poor cofactors for ATP hydrolysis (10). Low level binding to oligonucleotides of 8 nucleotides can only be detected in the presence of adenosine 5'-O-3-thiotriphosphate which causes tight binding to DNA (9). The binding site size on ssDNA is only 4 nucleotides for recA protein (12,13). This implies that protein-protein interaction is paramount for stable binding. The inability to detect binding to small oligonucleotides would suggest that recA protein has an extremely low intrinsic affinity for DNA; only protein-protein interactions would provide the necessary stability. This appears reasonable for a protein whose active form is a polymer.

In the presence of DNA, the hydrolysis of ATP has been shown to be cooperative (8). What is the origin of this cooperativity? One possibility is that binding to DNA is the only essential requirement. Protein-protein interactions would stabilize binding. Assuming that only recA protein

bound with ATP can provide this stability, then cooperativity would arise as an indirect effect. An alternative view is that in addition to providing stability, the hydrolysis of ATP on one monomer of recA protein directly induces the hydrolysis of ATP on an adjacent monomer. More complex schemes can also be described. A system which eliminates the DNA requirement for ATP hydrolysis would be valuable in elucidating the role of protein-nucleic acid interaction and protein-protein interaction in this process.

In this account we summarize work, described in detail elsewhere (14), on the kinetic mechanism of ATP hydrolysis by recA protein, using salts as functional analogs of DNA to simplify the system.

RESULTS

Small ions act as competitors of protein-nucleic acid interactions (15). Studies performed on recA protein in NaCl up to 0.5 M have revealed that DNA binding and thus the DNA-dependent ATPase activity was inhibited with increasing salt concentration (5). However, we observe that with higher salt concentrations the ATPase activity returns, but without a requirement for DNA. As shown in Table 1, many different salts were tested and all showed this property, although with different degrees of effectiveness.

The observed activity is probably a composite of known effects of salts on proteins in solution. However, salts which gave the highest activities in this system have been shown to also stabilize protein structure. When the ATPase activity was plotted as a function of molar salt concentration, a sigmoidal dependence was observed (data not shown) The dissociation constant for salt binding showed a third to fourth order dependence on salt concentration, depending on the type of ion. This type of analysis (15) suggested that at least three to four ions are taken up by recA protein to activate the ATPase. The effectiveness of various ions in stimulating the ATPase apppeared to follow the Hofmeister series (15,16). The Hofmeister series is a hierarchal arrangement of ions according to their effect on protein properties such as

solubility, thermal stability, enzyme activity, etc. (15,16). When comparing divalent ions such as magnesium acetate and sodium sulfate, a better fit to the series was obtained when ATPase activity was plotted as a function of anion concentration instead of cation concentration. On the basis of these results, we hypothesize that the observed activation of the recA protein ATPase is predominantly an anion effect.

TABLE 1
ATPase STIMULATION BY VARIOUS SALTS[a]

Salt	v/E[b]
	min^{-1}
none	0.02
sodium acetate	36
sodium glutamate	28
sodium chloride	22
pottassium chloride	31
magnesium acetate	20
ssDNA (poly dT)[c]	29
dsDNA (M13 mp8)[d]	22

[a]Reactions contained 50 mM Tris-HCl (pH 7.1), 5 mM ATP, 17.5 mM magnesium acetate, 5 uM recA protein, and 1.8 M of the indicated salt.

[b]Under these conditions v/E approximates k_{cat}, the apparent turnover number. v is the observed initial velocity, and E is the recA protein concentration.

[c]See Ref. 10 for conditions.

[d]See Ref. 17 for conditions.

For comparison, under conditions similar to those described in Table 1 (no salt), only micromolar concentrations of polydeoxythymidylate (poly dT), a long DNA homopolymer, are required to activate the ATPase. However, very small oligonucleotides are required in much higher

concentrations. For example, oligodeoxythymidylate which was four nucleotide residues in length was required at concentrations over 0.1 M to observe ATPase activity (data not shown). This suggests that the intrinsic affinity of recA protein for DNA and small ions are within an order of magnitude of each other.

The obvious requirement for protein-protein interaction on DNA for efficient ATP hydrolysis prompted us to determine if a similar requirement was maintained when in the high salt activated mode. As shown in Fig. 1, in the presence of 1.8 M NaCl, *v*/E exhibits a strong dependence on recA protein concentration when varied below 4 uM. This implies that aggregation of recA protein is necessary for ATP hydrolysis.

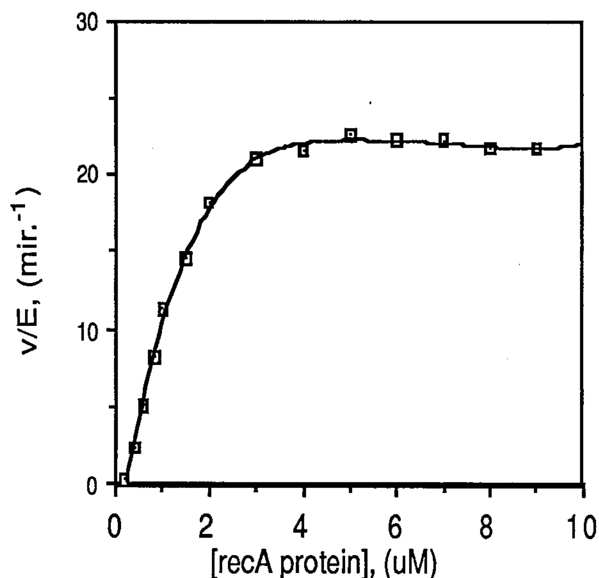

FIGURE 1. Dependence of recA protein ATPase on recA protein concentration in the presence of 1.8 M NaCl. See Table 1 for reaction conditions.

In the presence of DNA (no salt), magnesium is a necessary cofactor for ATP hydrolysis. In the high salt mode no ATP hydrolysis is observed in the absence of magnesium. However, as little as 1 mM magnesium acetate was sufficient to acheive 80% of the activity obtained under standard conditions described in Table 1 in the presence of 1.8 M NaCl.

DISCUSSION

The principle conclusion of the study summarized above is that the DNA-independent ATPase activity can be stimulated 2000 fold by the presence of high concentrations of salts. Many of the requirements of the reaction are similar to those found for the DNA-dependent reaction. These similarities are summarized in Table 2. Thus in many respects the high salt activation of the recA protein ATPase functionally mimicks the DNA-dependent reaction. One difference between the two systems is the lack of cooperativity with respect to ATP concentration in the high salt reaction. If one accepts the proposition that the two systems are mechanistically related, this can be taken as evidence that the cooperativity observed in the DNA-dependent reaction is a reflection of cooperativity in DNA binding rather than an interaction between the ATP binding sites in adjacent monomers.

TABLE 2
COMPARISONS WITH DNA-DEPENDENT ATPase

	High salt ATPase	DNA-dependent ATPase
cofactors	~4 anions	~4 nucleotides
k_{cat}	25-35 min^{-1}	25 min^{-1}
aggregation required	yes	yes
Mg^{2+} required	yes	yes
Hill coefficient	1.0	3.3

We do not know the mechanism of this activation, although many possibilities exist. The most intriguing one is that stimulation is produced by the specific binding of three to four anions at sites on recA protein that normally interact with the DNA phosphate backbone.

To our knowledge, this system is unique in the magnitude of stimulation afforded by salt. While obviously not physiological, this system represents a substantial decrease in complexity relative to DNA-dependent ATP hydrolysis, while retaining many of the important features of the reaction. Whether this phenomenon is specific to recA protein or if it may be generally applicable to other polynucleotide-dependent ATPases will be interesting to learn.

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