High Salt Activation of recA Protein ATPase in the Absence of DNA*

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The recA protein of *Escherichia coli* is a DNA-dependent ATPase. In the absence of DNA, the rate of recA protein-promoted ATP hydrolysis drops 2000-fold, exhibiting an apparent k_{cat} of approximately 0.015 min^{-1}. This DNA-independent activity can be stimulated to levels approximating those observed with DNA by adding high concentrations (approximately 2M) of a wide variety of salts. The increase in ATP hydrolysis appears to require the minimal interaction of three to four ions with recA protein. The active species in ATP hydrolysis is an aggregate of recA protein. There appears to be little or no cooperativity with respect to ATP binding (Hill coefficient = 1.0). The salt-stimulated ATP hydrolysis reaction is dependent upon Mg^{2+} ions and is optimal between pH 7.0 and 8.0. In many respects, the high salt concentration appears to be functionally mimicking DNA in activating the recA protein ATPase.

The recA protein of *Escherichia coli* promotes a DNA strand exchange reaction *in vitro* which provides a useful model for its role in homologous genetic recombination *in vivo* (for reviews, see Refs. 1 and 2). The active species in this reaction is a recA/DNA nucleoprotein filament (3, 4). recA protein binds ssDNA[1] cooperatively and unidirectionally, forming a highly ordered helical array (5–9). This complex then initiates the pairing of homologous DNA molecules and the exchange of identical strands of DNA (3, 4, 10–12). The initial pairing reaction requires ATP but not necessarily its hydrolysis (13, 14). After pairing, the subsequent unidirectional propagation of strand exchange or branch migration requires ATP hydrolysis (13, 15, 16). A number of models have been proposed which link ATP hydrolysis to branch movement (17, 18).[2] However, little is known about the details of this coupling. Previous studies (19–22) have shown that the recA protein ATPase requires a polynucleotide cofactor for ATP hydrolysis. recA protein hydrolyzes a variety of nucleotide triphosphates in addition to ATP (21, 23, 34). Each monomer contains one nucleotide triphosphate-binding site (25, 26).

The DNA-dependent ATPase activity has been studied

extensively (23, 27). Hydrolysis of ATP in the presence of ssDNA or dsDNA is pH-independent in the pH range of 6.0–9.0 (27, 28). Although earlier studies (27) have reported an apparent pH maximum on dsDNA at pH 6.2, recent studies (28) have shown that this is due to a kinetic barrier in the association process on dsDNA at pH 7.5. At steady state, recA protein promotes the same rate of ATP hydrolysis at pH 6.1 and 7.5 on dsDNA (28). ATP hydrolysis by recA protein exhibits a complicated dependence on ATP concentration, with Hill coefficients of about 3.3 at ATP concentrations below the K_m, for both ssDNA and dsDNA at its apparent pH optima (23). At ATP concentrations above K_m, both reactions exhibit a Hill coefficient of 1.0 (23).

The apparent K_m values for DNA-dependent ATP hydrolysis vary depending on the base composition of the DNA, temperature, recA protein concentration, etc. (23). For various ssDNAs, the K_m values range from approximately 20 to 100 μM, whereas for dsDNAs, they range from approximately 100 to 200 μM (23). The observed turnover number (k_{cat}) for ATP hydrolysis is approximately 25 min^{-1} for ssDNA (29) and 19–22 min^{-1} for dsDNA (28).

Previous reports have shown that ATP hydrolysis in the absence of a DNA cofactor occurs at only a fraction of the rate observed in the presence of DNA and has an apparent k_{cat} of 0.1 min^{-1} at pH 6, its pH optimum (23, 27). At pH 7.5, the k_{cat} for ATP hydrolysis in the absence of DNA is approximately 0.015 min^{-1} (23). Under these conditions, the recA protein ATPase is stimulated approximately 2000-fold by the presence of a DNA cofactor. This is reasonable for a protein whose primary function involves a DNA-dependent reaction.

recA protein exhibits substantial cooperativity in binding to a polynucleotide lattice (29–31). Single-stranded oligonucleotides with less than 50 nucleotides are poor cofactors for ATP hydrolysis (29), whereas the ssDNA-binding site size for recA protein is approximately four nucleotides (5, 30, 32, 33). This suggests that protein-protein interactions play an important role in binding to polynucleotides and stimulating the ATPase activity. On longer DNA molecules, recA protein binds stoichiometrically to form extended filaments (5, 34, 35). ATP is hydrolyzed throughout these filaments (29). Binding of recA protein to DNA appears to involve primarily interactions with the phosphate backbone of the DNA (36).

We now demonstrate that the activation of recA protein-promoted ATP hydrolysis by DNA can be mimicked by high (molar) concentrations of a number of salts. The resulting rates of ATP hydrolysis in the absence of DNA compare well with those observed under optimal conditions in the presence of DNA. We characterize this phenomenon and present it as a potentially simple model system in which to explore the mechanism of ATP hydrolysis by recA protein.

EXPERIMENTAL PROCEDURES

Materials—*E. coli* recA protein was isolated to greater than 99% purity as previously described (37). The concentration of recA protein

[1] The abbreviations used are: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; NaAc, sodium acetate; Mg(Ac)$_2$, magnesium acetate; MES, 2-(*N*-morpholino)ethanesulfonic acid; MOPS, 3-(*N*-morpholino)propanesulfonic acid; TAPS, 3-[tris-(hydroxymethyl)methyl]aminopropane sulfonic acid.

[2] M. M. Cox, B. C. Schutte, B. F. Pugh, S. W. Morrical, J. Lindsley, J. Lee, and J. Kim, manuscript in preparation.

in stock solutions was determined by absorbance at 280 nm, using an extinction coefficient of $\epsilon_{280} = 0.59\ A_{280}$ mg^{-1} ml. All enzymes and reagents (except the common salts) were purchased from Sigma.

Reaction Conditions—Unless otherwise noted, all reactions contained 50 mM Tris-HCl (80% cation, pH 7.1 at 37 °C), 17.5 mM Mg(Ac)$_2$, 5 mM ATP, 1.8 M NaCl, 2% (v/v) glycerol, 0.1 mM EDTA, 1 mM dithiothreitol, and 5 μM recA protein. For reactions using the enzyme-coupled spectrophotometric assay, approximately 100 units/ml pyruvate kinase, approximately 80 units/ml lactic dehydrogenase, 24 mM phosphoenolpyruvate, 2 mM KCl, and 3mM NADH were also included. All reactions were performed at 37 °C and were initiated by the addition of recA protein after preincubation of all other components at 37 °C for at least 10 min.

Enzyme-coupled Spectrophotometric ATPase Assay—This assay has been previously described (28, 38). Briefly, this coupling system employs pyruvate kinase, lactic dehydrogenase, and phosphoenolpyruvate to couple ATP hydrolysis to the conversion of NADH to NAD$^+$, which can be monitored spectrophotometrically by a decrease in absorbance at 380 nm. Due to the high concentrations of NADH used in this study, changes in absorbance were monitored at a wavelength of 380 nm, instead of 340 nm, its absorbance maximum, so as to remain in the linear region of the spectrophotometer. Because of the high concentrations of salt used in this study, the concentrations of coupling components listed above represent an increase relative to those previously described (28, 38). Subsequent to this analysis, it was found that different preparations of pyruvate kinase and pyruvate kinase from different sources gave substantially more activity. Although this had no effect on the results in this study, less of the coupling components may be required, depending on the preparation, in further studies. Unless otherwise noted, no component of the coupling system limited or interfered with the observed rate of ATP hydrolysis under all conditions of this study, as determined by the following criteria. When the concentrations of coupling components were increased, no increase in ATP hydrolysis was observed. When ADP (1 mM) was added to the reaction, it was rapidly converted to ATP with rates greater than 2 mM min^{-1}. When these criteria were not met, [^{3}H]ATP was employed (described below).

All absorbance measurements were obtained on a Perkin-Elmer Lambda 7 double beam recording spectrophotometer equipped with two six-position thermojacketed cuvette holders attached to a constant temperature water circulator. The slit width was 2 nm. Reactions (0.5 ml) were contained in self-masking quartz cuvettes with a path length of 0.5 cm. Values for ΔA_{380} were obtained by an enzyme kinetics software program installed in the spectrophotometer. Rates of ATP hydrolysis, expressed as micromolar ATP hydrolyzed to ADP per minute, were calculated from $-\Delta A_{380}$ min^{-1} obtained at steady state using an extinction coefficient of $\epsilon_{380} = 1210$ M^{-1} cm^{-1} for NADH. Changes in absorbance in the absence of recA protein were found to be small (equivalent to approximately 0.5 μM min^{-1} under standard conditions) and were subtracted from all data. Since ATP is constantly regenerated in this assay, the steady-state conversion of NADH to NAD reflects the initial rate of ATP hydrolysis at all times. Results obtained with this method were found to agree well with results obtained in assays employing [^{3}H]ATP (see below).

[^{3}H]ATP ATPase Assay—ATP hydrolysis was also measured by the conversion of [^{3}H]ATP to [^{3}H]ADP. ADP can be separated from ATP by chromatography on polyethyleneimine strips in 0.5 M formic acid and 1.0 M lithium chloride developing buffer as previously described (27). Since the high concentrations of salt in the reaction mixtures affected chromatography, samples were diluted 10-fold in water before applying them to polyethyleneimine strips. No ATP-regenerating system is used in these assays, so all coupling components described above were omitted. This assay was employed to confirm independently the results obtained using the assay described above and to obtain titration curves with salts that uncoupled (NaPO$_4$ (50% NaH$_2$PO$_4$ and 50% Na$_2$HPO$_4$)) or inhibited (Na$_2$SO$_4$ at high concentrations and reactions at low Mg^{2+} concentrations) the coupling system.

RESULTS

Dependence of ATP Hydrolysis on Salt Concentration—It has been observed that monovalent ions inhibit the DNA-dependent ATPase activity of recA protein (27). These studies were performed over a range of 0–0.5 M salt with increased inhibition as the salt concentration was raised from 0.1 to 0.5 M (27). However, as shown in Fig. 1, we observe that higher
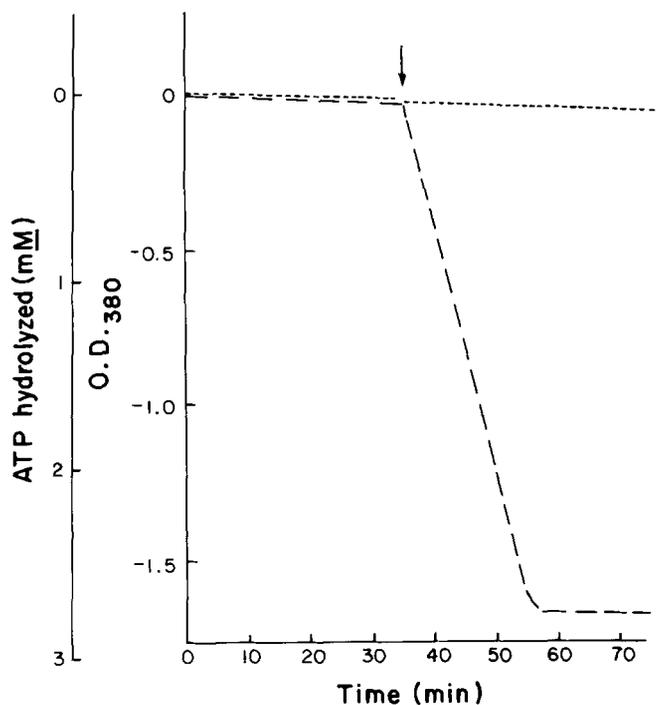

FIG. 1. **Stimulation of recA protein ATPase by high salt concentration.** Reactions were carried out at pH 7.1 with 5 mM ATP and 17.5 mM Mg(Ac)$_2$. Other conditions and the coupled assay for ATP hydrolysis are described under "Experimental Procedures," except that in one reaction (-----), no salt was added, and in the other (- - -), 1.6 M NaCl was added. Reactions were monitored spectrophotometrically for about 35 min without recA protein present. Following this, 10 μM recA protein was added (*arrow*) to each reaction. The absorbance (A_{380}) is plotted as a function of time. As shown, a ΔA_{380} of 0.6 unit corresponds to hydrolysis of 1 mM ATP. Complete conversion of NADH to NAD results in a ΔA_{380} of approximately 1.7.

salt concentrations stimulate ATP hydrolysis by recA protein in the absence of DNA. ATP hydrolysis was completely dependent upon the addition of recA protein, under all conditions. In this case, there is a marked enhancement in the reaction containing 1.6 M NaCl relative to the reaction containing no added salt.

This effect was further characterized by varying salt concentration and comparing the effects of different ion types. Ten different salts were tested (eight are shown), and most showed a dramatic stimulation of ATPase activity when present above 1.0 M (Fig. 2, *A* and *B*). Over the titration range tested (0–1.8 M), the activity showed a sigmoidal increase with salt concentration. The maximum ATPase activity was observed at the high salt end of the titration. NaAc and potassium acetate provided the highest activity, with apparent turnover numbers of 36 and 31 min^{-1} at 1.8 M salt, respectively. Under these conditions, the term $v/[E]$ (where v is the initial velocity and $[E]$ is the total enzyme concentration) approximates k_{cat}, the turnover number. As shown below, the ATP concentration is 5–10 times K_m, depending on salt concentration. Very high concentrations of salt inhibit the coupling system. In general, at concentrations above 2 M, the coupling system limited the observed rate of ATP hydrolysis. Under these conditions, the [^{3}H]ATP ATPase assay was used.

The origin of this salt activation is unlikely to be a general ionic strength effect since the different salts show vastly different levels of activation at the same ionic strength. For example, at an ionic strength of 1.6 M, the apparent turnover numbers in the presence of NaAc and Na$_2$SO$_4$ (as the main contributors to the ionic strength) are 34 and 2 min^{-1}. The
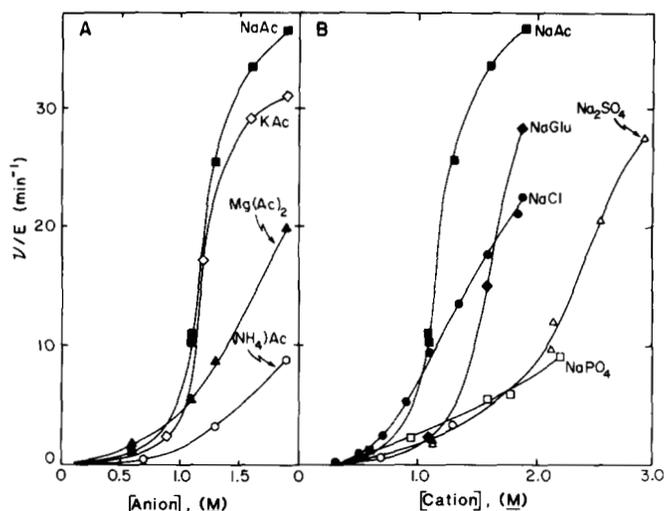
FIG. 2. **Dependence of recA protein ATPase on concentration of various salts.** Reactions were carried out at pH 7.1 with 5 μM recA protein, 5 mM ATP, and 17.5 mM Mg(Ac)$_2$. Other reaction conditions are described under "Experimental Procedures," except that the salt concentration was varied as shown. In *A*, the anion (acetate) was held in common, whereas in *B*, the cation (Na$^+$) was. *KAc*, potassium acetate; *(NH$_4$)Ac*, ammonium acetate; *NaGlu*, sodium glutamate.

observed activation appears to result from a net contribution of anions and cations. At 1.8 M acetate, the observed stimulation showed the following cation trend: Na$^+$ > K$^+$ > Mg^{2+} > NH$_4^+$ (Fig. 2*A*). At 1.8 M Na$^+$, the following anion trend was observed: Ac$^-$ > Glu$^-$ > Cl$^-$ > SO$_4^2$ > PO$_4$ (Fig. 2*B*). These trends probably bear no special significance beyond the fact that they illustrate the ion-specific nature of the activation. When compared at lower Na$^+$ or acetate concentrations, the trends in relative activity of different salts were somewhat altered.

The observed activation is likely to have multiple contributions. Ions such as phosphate and magnesium are known to interact specifically with recA protein (27, 32). In addition to its role in ATP hydrolysis, a low concentration of magnesium induces the aggregation of recA protein when ATP is absent (32). Phosphate is a product of the ATP hydrolytic reaction (27) and so may affect the activity when present at high concentrations. Salts in general have a number of demonstrated effects on protein properties, mediated either by direct global binding of ions to the protein or indirectly through effects on the solvent (39–47). In general, these properties, which include solubility, hydration, melting temperature, partial specific volume, etc., are affected similarly by specific ions such that the ions can be arranged in a hierarchy (Hofmeister series) reflecting their effectiveness (39–47). The salts used in this study fall at one end of the series, those which are preferentially excluded from the domain of the protein (39–47). This exclusion causes the protein to be preferentially hydrated, tending to become more compact so as to release locally bound water to the solvent (40, 46). If the activation is the result of preferential hydration of the protein, then activation should follow a pattern consistent with the Hofmeister series (39–47). Furthermore, sugars such as glucose should be equally effective in activating the ATPase since they too cause preferential hydration of proteins (48, 49). The activation pattern in Fig. 2 fails to show a pattern consistent with the Hofmeister series, and concentrations of glucose that preferentially hydrate proteins fail to stimulate the ATPase (data not shown). Therefore, it seems unlikely that activation is solely a result of a global nonspecific inter-

action of ions with the protein or a release of protein-bound water. Salt effects have been studied with other enzymes and proteins (39–47). Most of the characterized salt effects tend to cause a decrease in enzymatic activity (39, 40). A variety of demonstrated effects of salts on protein hydration, structure, etc. are likely to be present in this system and may affect the net observed activation. The large observed activation, however, is unusual and may represent a unique interaction of ions with recA protein. One possibility is that activation is caused by a direct binding of the ions to the protein. Since this is inconsistent with the demonstrated noninteractive nature of these ions, one would have to invoke low affinity-specific ion-binding sites on the protein that become saturated at very high salt concentrations.

We wished to determine more quantitatively the nature of this effect. If one assumes that the stimulation reflects the existence of two conformations of recA protein (R), one containing *m* specifically bound ions (S*), which are required for ATP hydrolysis, then the equilibrium constant K for R $\rightleftharpoons$ RS*$_m$ can be defined as $K = [\text{RS*}_m]/[\text{R}] = \nu/(V - \nu)$. The steady-state velocity of ATP hydrolysis (ν) is a measure of the ion-bound form. $V - \nu$ is a measure of the concentration of inactive recA protein, where V is the theoretical limit of ν at infinite salt concentration. The net change in the number of anions, cations, and water thermodynamically bound to the protein is directly related to the change in log K as a function of the change in log [salt]. This type of analysis has been described in detail by Record *et al.* (45).

In applying this approach to our system, a number of simplifications and assumptions are made. 1) Activity coefficients are not included in the analysis. This may result in an underestimation of the change in the number of bound ions. 2) The contribution of any change in the number of bound water molecules is assumed to be negligible. This assumption is based on the observation above that water release does not appear to be a major driving force in the reaction. 3) Since ATP hydrolysis was monitored, we assume that the observed change in the state of the protein reflects only those ions which participate directly in activating the ATPase. Clearly, a variety of additional salt effects (see above and "Discussion") could alter the observed rate. These secondary effects may be responsible for the different activities of each salt. Therefore, we assume that this analysis would provide only an estimate of the minimum number of ions required to activate the ATPase. 4) Inhibition of the coupling enzymes and other experimental problems limit the accuracy of data obtained at very high salt concentrations. For these reasons, V, and thus $\nu/(V - \nu)$, cannot be determined accurately. If the data are plotted as log ν instead of log $\nu/(V - \nu)$, then a linear relationship would be observed only at values of ν where $V - \nu \approx V$. In this region, K is proportional to ν, with V as a proportionality constant. Significant nonlinearity would occur at higher values of ν. To avoid the problem of determining V, data are plotted as log ν *versus* log [salt] only for lower values of ν which follow a linear relationship. The slopes of these plots are interpretable as the net change in the number of thermodynamically bound ions and water which influence the ATPase activity.

Plots of log ν *versus* log [salt] for three of the salts from Fig. 2 are presented in Fig. 3. Slopes of 3.0 for NaCl, 3.2 for sodium glutamate, and 3.9 for NaAc are obtained. If the uptake of ions is required to stimulate the recA protein ATPase, then the thermodynamic equivalent of three to four ions becomes bound. Although other explanations cannot be ruled out, this appears to be the simplest interpretation. The high concentrations of salt required for activation indicates
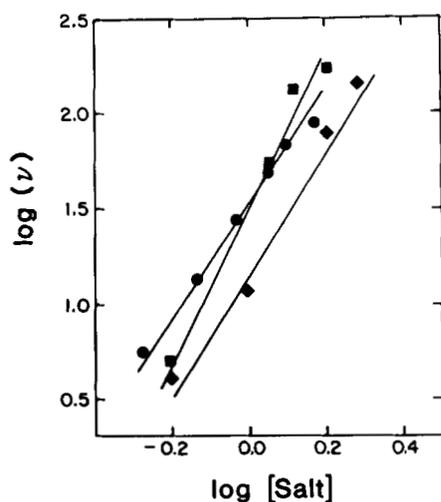
FIG. 3. **Order of dependence on salt concentration.** The titration data from Fig. 2 for NaCl (●), sodium glutamate (◆), and NaAc (■) are plotted on a log scale. The slopes of the lines drawn are 3.0, 3.2, and 3.9, respectively. See text for details.

that the dissociation constant for ions involved in the activation is in the molar range. As described below, the intrinsic dissociation constant of recA protein for ssDNA also appears to be very high.

Binding of recA protein to a polynucleotide is a highly cooperative process (29–31). As a result, the apparent dissociation constant (K_d) of a recA-polynucleotide complex has contributions from both K_i, the intrinsic dissociation constant to the polynucleotide, and ω, the cooperativity paramenter (50, 51). By varing the polynucleotide length over the appropriate range, ω can be altered systematically (29). Brenner *et al.* (29) examined K_d as a function of single-stranded DNA length and found that when the DNA length was reduced from 50 nucleotides to 25 nucleotides, K_d increased from 5.6 to 333 μM. Below this length, very high concentrations of oligonucleotides were required for activity. The single-stranded DNA-binding site size for recA protein is only ~four nucleotides (5, 30, 32, 33). This suggests that K_i may be extremely large. We investigated this length dependence further using a 4-base oligonucleotide. The preliminary results show that oligonucleotide concentrations greater than 100 mM were required to obtain significant activity (not shown). This indicated that the intrinsic dissociation constant for ssDNA may be very high, perhaps in the molar range. For the high salt activation, the intrinsic dissociation constant for salt binding also appears to be in the molar range.

As shown below, aggregation of recA protein appears necessary for ATP hydrolysis. Although the above analysis is described for a monomer, it is equally valid for a recA protein aggregate. The numbers cited above may therefore reflect the ions required for activation of a recA aggregate rather than an individual monomer.

Dependence on recA Protein Concentration—ATP hydrolysis in the presence of DNA is a cooperative process, requiring the interaction of adjacent monomers on a DNA lattice for efficient ATP hydrolysis (23, 29). To determine if a similar requirement is maintained in the absence of a DNA cofactor, recA protein ATPase activity was monitored over a range of recA protein concentrations at three different NaCl concentrations (Fig. 4A). In these titrations, the ATP concentration was maintained at about 4–6 times higher than K_m. Below 5 μM recA protein, $v/[E]$ exhibits a strong dependence on recA protein concentration. The order of this dependence increases with NaCl concentration. This suggests that self-association
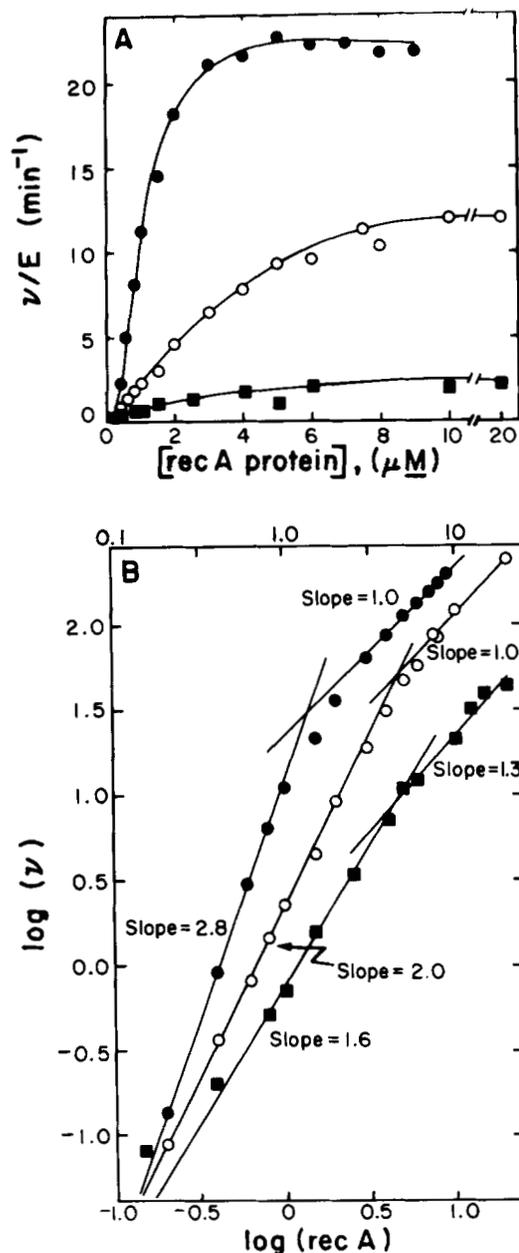

FIG. 4. **Dependence of recA protein ATPase on recA protein concentration.** Reactions were carried out at pH 7.1 with 5 mM ATP, and 17.5 mM Mg(Ac)₂. Other reaction conditions are described under "Experimental Procedures," except that the recA protein concentration was varied in reactions containing 0.5 M (■), 1.0 M (O), or 1.8 M (●) NaCl. *A*, recA protein titration curves *versus* apparent turnover number; *B*, log-log plot of the initial velocity *versus* recA protein concentration (micromolar). Data and symbols are the same in *A*.

of recA protein is necessary for ATP hydrolysis.

In an analysis similar to that described above for the uptake of ions by recA protein, a minimal estimate of the aggregation size can be determined. If one assumes an equilibrium between a monomeric form of recA protein (R) and an aggregated form (R_n), containing n monomers minimally required for ATP hydrolysis, then the equilibrium constant K for $nR \rightleftharpoons R_n$ can be defined as $K = [R_n]/[R]^n$, where $[R_n]$ is defined in units of monomers. The fraction in the catalytically active form (θ) is given by $\theta = [R_n]/[R_t]$, where $[R_t]$ is the total enzyme concentration. V is not a direct measure of aggregation in this analysis since it varies with enzyme concentration. However,

at high enzyme concentration, $v/[R_t]$ (or $v/[E]$) is nearly constant and can be defined as k_{cat} at the theoretical limit of infinite enzyme concentration. Thus, $\theta = [R_n]/[R_t] = (v/[R_t])/(k_{cat})$. This reduces to $v/k_{cat} = [R_n] = K[R]^n$, and in logarithmic form, $\log v = \log Kk_{cat} + n \log [R]$.

In Fig. 4B, plots of $\log v$ *versus* $\log [R_t]$ are shown. At low recA protein concentration, where $[R] = [R_t]$, the slopes (n) are interpretable as the minimal aggregation size necessary for optimal ATP hydrolysis. At 1.8 M NaCl, a slope of 2.8 is observed. This value decreases to 2.0 and 1.6 at 1.0 and 0.5 M NaCl, respectively. At very low velocities (below $\log v = -0.5$), the data are subjected to considerable error, thereby limiting interpretation. In this analysis, we assume that recA protein exists in an equilibrium between monomers and an aggregated form. However, this does not preclude an equilibrium of small aggregates with a larger aggregate. Therefore, these values represent a lower limit of the critical aggregate size required to hydrolyze ATP. Additional interpretations of these data are possible as described under "Discussion."

At high recA protein concentration, the slopes are approximately 1.0. Therefore, the initial velocity is directly propor-
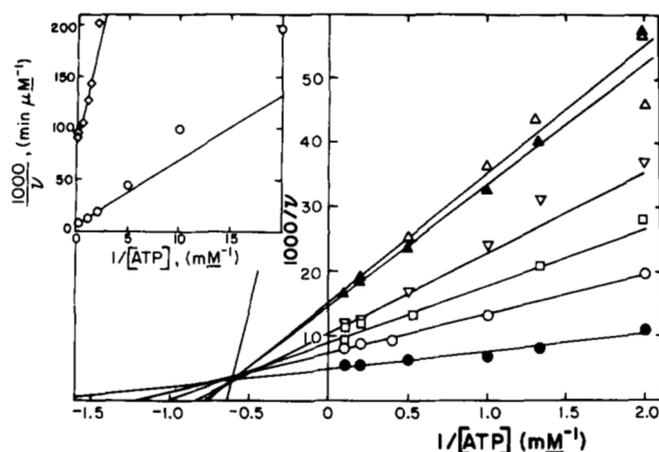

FIG. 5. **Lineweaver-Burk plot of effect of ATP concentration on recA protein ATPase.** Reactions were carried out at pH 7.1 with 5 μM recA protein and 17.5 mM Mg(Ac)$_2$. Other reaction conditions are described under "Experimental Procedures," except that ATP concentrations were varied as shown. Salt identities and concentrations are: $\triangle$, 1.0 M NaCl; $\blacktriangle$, 1.0 M NaAc; $\triangledown$, 1.2 M NaCl; $\square$, 1.4 M NaCl; $\bigcirc$, 1.8 M NaCl; $\bullet$, 1.8 M NaAc. *Inset*: $\diamondsuit$, 0.5 M NaCl, $\bigcirc$, 1.8 M NaCl (on an expanded scale). The extrapolated line for the 0.5 M NaCl data is shown in the vicinity of the crossover point. The coordinates for these plots were determined as described for Table I, using these and additional data points.

tional to total enzyme concentration, and self-association does not contribute significantly to the observed rate under these conditions. The recA protein concentration used for the salt titrations shown in Fig. 2 was high enough to fall in this first-order range. The observed dependence of $v/[E]$ on salt concentration shown in Fig. 2 is therefore not a result of a change in reaction order, *i.e.* self-association was not limiting the observed velocity. The results shown in Fig. 4B indicate that salts can have two distinct effects on recA protein: to increase V_{max} for ATP hydrolysis and to increase the apparent reaction order with respect to recA protein concentration.

Dependence of ATP Hydrolysis on ATP Concentration— The dependence of the recA protein ATPase activity on ATP concentration was examined at fixed levels of NaCl or NaAc. The data are presented as double reciprocal plots in Fig. 5. The intersecting pattern indicates that both ATP and ions bind to the enzyme before products are released. The horizontal coordinate of the crossover point represents $-1/K_d^{ATP}$, where K_d^{ATP} is the dissociation constant of the binary recA-ATP complex (52). The K_d^{ATP} derived from these data is 1.85 mM over the range of 0.5–1.8 M NaCl or NaAc tested. The kinetic parameters are summarized in Table I. Both V_{max} and V/K_m increase with increasing salt concentration. This suggests that ion binding affects one or more steps leading up to and including ATP hydrolysis or release of the first product.

Fig. 6A shows an ATP titration of the ATPase activity at 1.8 M NaCl. These and additional data points were used to construct a Hill plot shown in Fig. 6B. The Hill coefficient is 1.0 over the range of 0.002–10.0 mM ATP. This suggests that little or no cooperativity exists in this system with respect to ATP binding. The Hill coefficient remains essentially unchanged if the salt concentration is reduced to 0.5 M (data not shown).

Dependence of ATP Hydrolysis on Mg^{2+} Concentration— DNA-dependent ATP hydrolysis by recA protein requires Mg^{2+} as a cofactor. To determine its requirement for the salt-stimulated reaction, an Mg^{2+} titration was performed in the presence of 1.8 M NaCl (not shown). When Mg^{2+} was omitted from the reaction, no ATP hydrolysis was observed. However, as little as 1 mM Mg^{2+} was sufficient to regain 80% of the activity in the presence of 5 mM ATP under otherwise standard reaction conditions.

Dependence of ATP Hydrolysis on pH— The DNA-independent recA protein ATPase activity has been previously shown to be optimal near pH 6.0. To establish whether this held true when in the salt-activated mode, pH titrations were performed in the presence and absence of 1.8 M NaCl. As shown in Fig. 7, distinctly different profiles were obtained.

TABLE I

Kinetic parameters for stimulation of recA protein ATPase by high salt

Kinetic parameters and standard errors were obtained by fitting data shown in Fig. 5 to the Michaelis-Menten equation $v = V[A]/(K_m + [A])$ using the FORTRAN computer program HYPERO written by Cleland (53). v represents a measured reaction velocity, V or V_{max} is the maximum velocity at the specified salt concentration, K_m is the Michaelis constant for ATP, and [A] is the ATP concentration. $k_{cat(app)}$ is the apparent turnover number and is equal to $V/[E]$, where E is the total enzyme concentration.

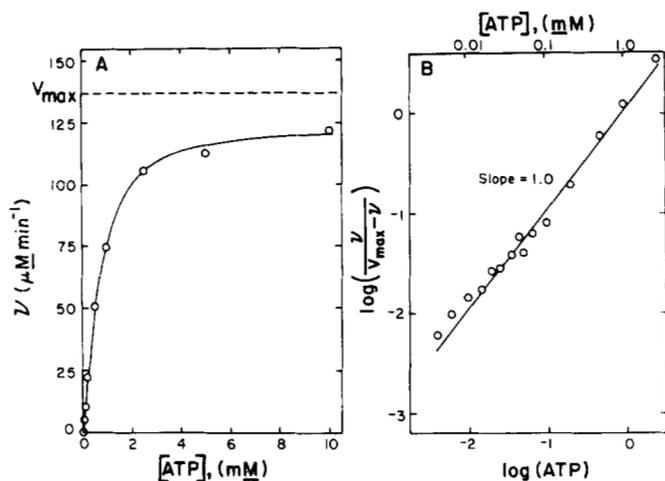| Salt	[Salt]	V_{max}	K_m	V/K_m	$k_{cat(app)}$
	M	$\mu M\ min^{-1}$	μM	min^{-1}	min^{-1}
NaCl	0.5	14 ± 0	1530 ± 80	0.009 ± 0.000	2.8
	1.0	68 ± 3	1340 ± 150	0.051 ± 0.004	13.6
	1.2	97 ± 4	1340 ± 150	0.073 ± 0.006	19.4
	1.4	113 ± 4	1010 ± 130	0.112 ± 0.011	23.6
	1.8	134 ± 4	830 ± 80	0.161 ± 0.012	26.8
NaAc	1.0	69 ± 1	1310 ± 100	0.053 ± 0.002	13.8
	1.8	190 ± 9	420 ± 90	0.450 ± 0.077	38.0

FIG. 6. **Hill plot of effect of ATP concentration on recA protein ATPase.** Reactions were carried out at pH 7.1 with 17.5 mM Mg(Ac)$_2$, 1.8 M NaCl, and 5 μM recA protein. Other reaction conditions are described under "Experimental Procedures," except that the ATP concentration was varied as indicated. *A*, ATP titration curve. These data were also used in a Lineweaver-Burk plot (1.8 M NaCl) presented in Fig. 5. *B*, Hill plot using the data from *A* and additional data. Data were fit by linear regression.

The *dashed line* drawn near the base of *A* represents the scale of *B*. At high salt, the pH optimum was in the pH range of 7–8.5. Under low salt conditions, the activity was optimal near pH 6.0, confirming previous results (27). The basis of this shift in the apparent pH optimum at the high salt concentration is not clear.

DISCUSSION

The principle conclusion of this study is that the DNA-independent ATPase activity of recA protein can be stimulated 2000-fold by the presence of high concentrations of salts. This stimulation exhibits a third- to fourth-order dependence on salt concentration. ATP hydrolysis requires the interaction of recA monomers, exhibiting nearly a third-order dependence on recA protein concentration at high salt. There is little or no cooperativity with respect to ATP binding. The reaction requires Mg^{2+} and exhibits a pH optimum between pH 7 and 8.5.

Mechanism of Salt Activation—An apparently similar salt activation has been reported for the dynein-I ATPase from sea urchin sperm flagella (54). In those studies, maximum stimulation occurred between 0.6 and 0.8 M salt, resulting in as much as a 20-fold increase in activity over that in the absence of salt. The stimulation was observed only with salts which tend to destabilize protein tertiary structure. The mechanism of activation in this case is probably unrelated to that of recA protein since we observe the opposite effect: activation with structure-stabilizing ions. Furthermore, dynein-I ATPase activity could be stimulated by low concentrations of Triton X-100. Similar amounts of this detergent totally inactivated the activity of recA protein (data not shown).

The effects of very high salt concentrations have been examined for other proteins including myosin nucleotide triphosphatase, lactic dehydrogenase, fumarase, and estradiol-17β dehydrogenase (39). The principle conclusion of those studies was that cations and anions could be categorized according to their effectiveness in either protecting or destabilizing the tertiary structure of the protein. The arrangement generally followed the Hofmeister series (39, 42, 43). However, of the salts tested, none increased the activity of the enzyme relative to activity in the absence of added salt; most decreased
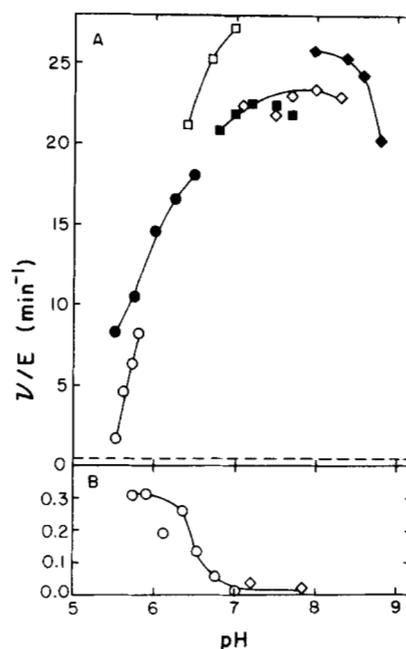

FIG. 7. **Dependence of recA protein ATPase on pH.** Reactions contained 5 mM ATP and 5 μM recA protein. Other reaction conditions are described under "Experimental Procedures," except that the pH was varied. Assays were performed in the presence (*A*) or absence (*B*) of 1.8 M NaCl. The pH was varied with HCl for the cationic buffers and with NaOH for the anionic buffers. Buffers used are maleate (O), MES (●), imidizole (□), MOPS (■), Tris (◇), and TAPS (◆). In the titration shown in *B*, constant ion concentration was maintained with NaCl.

the activity (39), as evidenced by the coupling enzymes employed in this study. As with those enzymes, we would expect recA protein to respond similarly, having the same or less activity depending upon the salt. The salts used in our study are considered protecting ions and thus should maintain the globular shape of the protein as well as its activity. The exact nature of these particular salt effects is unknown, although models have been proposed which invoke a global, nonstoichiometric interaction of destabilizing salts with the protein, resulting in a displacement of bound water and an unfolding of the tertiary structure (43, 44). Protecting salts are thought to be preferentially excluded from the domain of the protein, resulting in a preferential hydration of the protein (40, 46). As a result, the protein tends to minimize its solvent exposure by becoming more compact and thus more resistant to unfolding (40, 46). It is not obvious from these general effects how protecting salts would produce such a large (2000-fold) stimulation of ATP hydrolysis.

Although it is likely that both protecting and destabilizing salts would affect recA protein in a manner similar to that of other proteins, there must be an additional mode of interaction which gives rise to the very large stimulatory effect. Although the ions used in this study tend to be excluded preferentially from the domain of the protein, the most intriguing possibility is that stimulation is produced by binding of three to four ions (possibly anions) at specific sites on recA protein. Such sites, for example, may be the DNA-binding site which normally interacts with the DNA phosphate backbone. As with DNA, this ionic interaction activates the ATPase. We note that the system described here has several properties which identify it with DNA-dependent ATP hydrolysis. If the dependence on salt concentration reflects an effect on each recA monomer, then the apparent requirement for three to four ions corresponds closely with the binding

site size for recA protein on ssDNA. Leahy and Radding (36) have reported that recA protein is bound to DNA along the phosphate backbone, and our results suggest that the salt activation is functionally mimicking the ionic interaction of the protein with DNA. In addition, the reaction exhibits rates of ATP turnover at least as high as those observed in the DNA-dependent reaction. The reaction requires the interaction of recA monomers, as is the case on DNA. Finally, the intrinsic dissociation constants for ssDNA and salts both appear to be in a similar range.

Mechanism of ATP Hydrolysis—The active species in ATP hydrolysis is an aggregate of recA protein (23). As shown in Fig. 4, the order of this apparent association stoichiometry decreases with decreasing salt. One interpretation of this result is that salt increases the dissociation constant of the recA aggregate. At low salt (≤0.5 M), recA protein remains stably self-associated through successive cycles of ATP hydrolysis, resulting in an apparent first-order dependence of velocity on recA protein concentration. As the salt concentration increases, the complex becomes more unstable and dissociates either partially or fully during the hydrolytic cycle. The apparent reaction order increases with increasing salt until complete dissociation into monomers occurs with each cycle. At the highest salt concentration tested, a 2.8-order dependence on recA protein was observed. This suggests that the minimum size of the active ATPase is approximately three monomers, but may be much larger. At recA protein concentrations well above the aggregate dissociation constant, recA protein is predominantly in the associated state, and first-order dependence is observed at all salt concentrations. If recA protein aggregates remain intact through the ATP hydrolytic cycle at low salt, then ATP binding does not require free recA monomers. Hill coefficients of 1.0–1.2, observed at 0.5 and 1.8 M NaCl, suggest that little or no cooperativity exists in ATP binding and hydrolysis. A second and equally plausible interpretation of these aggregation results is that the minimum size of the recA protein aggregate required for ATP hydrolysis changes as a function of salt. For example, with increasing salt concentration, monomers, then dimers, and then trimers, etc. become the minimal unit for ATP hydrolysis, and the aggregation state contributes significantly to the observed kinetics at low recA protein concentration.

A summary of the reaction scheme in its simplest form is illustrated in Fig. 8. Many of the steps are tentative. We assume that the active species is an aggregate and that three to four ions are required to activate each monomer within the aggregate. This scheme focuses on the ATP hydrolytic cycle of one monomer ($S^*_mRS_p$), which contains m specifically bound anions (S^*) required for ATP hydrolysis, and p ions (S) which are released upon the self-association of n recA monomers (R). These two effects are distinct. The other recA monomers presumably also bind S^* and ATP (A) and release S, but for simplicity are designated as R. If our first interpretation (see above) of the data related to the aggregation state is correct, then step 1 does not require ATP. However, this does not preclude a random binding of ATP to the monomeric

or aggregated form. ATP binding (step 2), hydrolysis (step 3), and product, P_i, and ADP (D) release (step 4) are also shown.

To our knowledge, this system is unique in the magnitude of the stimulation afforded by salt. Whereas the conditions employed are clearly not physiological, the system may provide advantages for basic studies of ATP hydrolysis by recA protein. In particular, it represents a substantial decrease in complexity relative to DNA-dependent ATP hydrolysis while retaining many of the important features of that reaction. Although this study provides some of the details of the reaction sequence, additional studies are necessary to determine the precise aggregation state of recA protein at each salt concentration, the sequence of some of the steps outlined above, and kinetic rate constants for each. It will be interesting to determine if this phenomenon is specific to recA protein or if it may be generally applicable to all polynucleotide-dependent ATPases.

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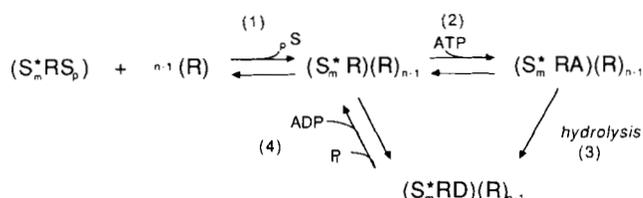

FIG. 8. **A model for salt-stimulated ATP hydrolysis by recA protein.** See text for details.

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