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DNA SEQUENCE DEPENDENCE OF ATP HYDROLYSIS BY RECA PROTEIN

Mohan Amaratunga and Albert S. Benight*

Department of Chemistry, University of Illinois at Chicago
Chicago, Illinois 60680

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SUMMARY: The DNA sequence dependence of the ATPase activity of RecA protein has been investigated for a variety of single strand octamer and hexadecamer homopolymers and alternating copolymers. Under assay conditions where the single strand DNA concentration exceeds the RecA protein concentration, significant differences in the rates of ATP hydrolysis for the various single strand DNA oligomer cofactors are observed. Under the conditions examined, the order of efficiency of the DNA cofactors in inducing RecA mediated ATPase activity is found to be: $dA_{16} > dT_{16} > d(TC)_{16} > dT_8 > dC_{16} > dA_8 = dG_8 > dG_{16} > dC_8 > d(AG)_{16}$. These results demonstrate not only a dependence of RecA ATPase activity on the sequence composition of short single strand DNA they further reveal ATPase activity can be affected by the nearest neighbor nucleotide sequence of short DNA cofactors.

In *Escherichia coli*, the machinery of both general genetic recombination and post replicative repair of DNA damage require RecA protein (1,2). Intrinsic to both these rather crucial cellular processes RecA exhibits two particular enzymatic activities that require both ssDNA and nucleoside triphosphates such as ATP. In one of these, RecA protein catalyzes exchange of complementary strands between separate DNA molecules in an ATP dependent reaction thought to be the *in vitro* analog of homologous genetic recombination in vivo (3-5). In the other, constitutive RecA protein is activated to become a specific proteolytic enzyme when cellular DNA damage is incurred by ultraviolet radiation or deleterious chemical agents. In this latter mode RecA protein cleaves the LexA repressor thereby derepressing its own structural gene and at least 20 other genes whose products are involved in DNA repair (the pleiotropic SOS response)(2). Nearly all the reactions mediated by RecA activity *in vitro* require the presence of ATP in the reaction mixture, however, ATP hydrolysis does not appear to be a concomitant requirement in all cases.

*Address Correspondence to this Author
Abbreviation: single strand DNA, ssDNA

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In the sequence of events mediated by RecA which lead to strand exchange, the presence of ATP but not ATP hydrolysis is required for initial pairing of RecA coated ssDNA with homologous double strand regions to form the so called ternary complex (8,9). Subsequent propagation of strand exchange through branch migration does require ATP hydrolysis (9-11). Although several plausible models relating the hydrolysis of ATP to branch migration have been presented (12,13), the precise mechanism under which branch migration and ATP hydrolysis are coupled remains to be illusidated.

In vitro, under physiological solution conditions, the conversion of RecA protein to its proteolytically active form requires both ssDNA and ATP in the reaction mixture but hydrolysis of ATP is not essential for repressor cleavage (14,15). The primary interaction of ssDNA and ATP cofactors in the RecA catalyzed cleavage of both the Lex A and lambda repressors is primarily with the RecA protein and not the repressor proteins (14). RecA also displays a ssDNA dependent ATPase activity (16-18). Similarities between the DNA cofactors required to activate RecA protease and ATPase activity suggest a common mode of interaction of the DNA and ATP with RecA is responsible for both types of enzymatic behavior (14). For this reason and because of the requirement of ATP hydrolysis by RecA in strand exchange, the DNA dependent ATPase activity of RecA protein has been the focus of extensive study (16-21).

In this paper we investigate the DNA sequence dependence of RecA ATPase activity under reaction conditions where the ssDNA concentration of homopolymers eight and sixteen nucleotides in length (48 μM base nucleotides) exceeds the RecA protein concentration (800 nM RecA). Although this protein/DNA molar ratio and ssDNA substrate length have been reported to poorly support RecA/DNA complexation or induce RecA ATPase activity, significant differences in the rates of ATP hydrolysis for the various DNA homopolymer substrates are observed.

MATERIALS AND METHODS

DNA

The ssDNA oligomers listed in Table I were synthesised on an Applied Biosystems 380B DNA synthesizer using the standard β-cyanoethyl phosphoramidite chemistry (22). After deprotection, each oligomer was purified to homogeneity electrophoretically on DNA denaturing gels (16% polyacrylamide, 7M Urea). DNA was eluted from gel slices by incubating each slice in buffer (100 mM NaCl, 25 mM Tris-HCl, pH=7.2) for 24 hours at 60 ^{o}C. Eluted DNA was then run over a hydroxylapatite column. Purified oligomers were demonstrated by electrophoresis to be greater than 99.5% homogeneously pure. Followed by desalting on a Sephadex G-50 column DNA oligomers were lyophilized and stored at -20 ^{o}C. Prior to experiments each oligomer was brought up in one ml of double distilled water. Concentration of the stock DNA solutions was between 5 and 10 mM (base nucleotides). Concentrations of the oligomers were determined from the absorbance at 260 nm using the extinction coefficients given in Table I calculated by the nearest neighbor method (23).

Protein

Purified RecA protein was the generous gift of Dr. Stephen Brenner, Central Research and Development Department, E.I. du Pont de Nemours, Wilmington, Delaware. Prior to use in the experiments purity of the protein was verified by SDS polyacrylamide electrophoresis. The extinction coefficient used in determining the protein concentration was ϵ_{280} = 2.32 x 10^4 $M^{-1}cm^{-1}$ at 280 nm.

ATPase Assay

ATPase activity was measured according to the procedure previously described (24-26). In this assay pyruvate kinase, lactic dehydrogenase and phosphoenol pyruvate directly couple the hydrolysis of ATP to ADP and P_i to the oxidation of NADH to NAD^+ which can be monitored spectroscopically. Under steady-state conditions the rate of ATP hydrolysis is directly proportional to the rate of the absorbance decrease observed at 340 nm. Assays were performed on a Hewlett Packard 8450A double beam spectrophotometer. All components of the coupling system were purchased from Sigma, St. Louis, MO. The reaction mixture contained 1 mg of NADH dissolved in 10 ml of the reaction buffer (20 mM Tris-HCl, pH=7.5, 100 μM DTT, 1 mM ATP, 2 mM phosphoenol pyruvate, 4 mM $MgCl_2$). In a reaction volume of 1 ml, 12.5 units each of pyruvate kinase and lactic dehydrogenase were added and equilibrated for two minutes at 37 ^oC. Reactions were initiated by adding RecA protein to a final concentration of 800 nM and the appropriate DNA oligomer to a final concentration of 48 μM (base nucleotides). The ATP hydrolysis rate was determined from the slope of the linear region of the hypochromicity versus time curve with the internal software of the spectrophotometer using the dA/dt key (26). All assays were performed at 37 ^oC. For each DNA oligomer at least two experiments were performed. Calibration of the assay was verified by using a 3 μM concentration (base nucleotides) of M13mp19 ssDNA as the cofactor which produced an ATP hydrolysis rate virtually identical to that previously reported for an M13 cofactor (25). Under the conditions employed in the assay, ATP hydrolysis is rate limiting.

RESULTS

Results of the ssDNA dependent ATPase assays, measured at constant DNA and RecA protein concentrations, are summarized in Table I and depicted in Figure I. Relative rates of ATP hydrolysis, $R_{rel} = k_{DNA}/k_0$, are given where k_{DNA} and k_0 are the ATP hydrolysis rates at 37 ^oC in the presence and absence, respectively, of DNA cofactor. $k_0 = c \times (3\mu M \text{ ATP/sec})$, where c is the proportionality constant relating the hypochromicity at 340 nm versus time to the absolute rate of ATP hydrolysis. The confidence interval, σ, of R_{rel} given in Table I provides an indication of the reproducibility in determining the ATP hydrolysis rate for each ssDNA cofactor.

Results indicate that under the employed conditions every ssDNA cofactor, to some extent, enhances RecA ATPase activity above the DNA independent rate. The most efficient DNA cofactor of the set, dA_{16} (R_{rel}=10) induces an ATP hydrolysis rate nearly eight times that of the poorest cofactor $d(AG)_{16}$ (R_{rel}=1.3). For the poly(dA), poly(dT) and poly(dC) oligomers a definite length dependence of R_{rel} is observed and the R_{rel}'s of dA_{16}, dT_{16} and dC_{16} are greater than those of dA_8, dT_8 and dC_8 by factors of 4, 2.4 and 1.6, respectively, although dT_8 is a better cofactor for the

Table I : DNA cofactors and RecA ATPase activity*

DNA Oligomer	Extinction Coefficient (ε_{260} x10^{-4} M^{-1} cm^{-1})	(i) (ii) $-\frac{dA}{dt} = \frac{k_{DNA}}{c}$	(iii) $R_{rel} = \frac{k_{DNA}}{k_0}$	(iv) $\pm\sigma\ (R_{rel})$
dA_{16}	1.54	30.5	10.0	0.2
dC_{16}	0.74	8.2	2.7	0.1
dG_{16}	1.15	6.1	2.0	0.08
dT_{16}	0.87	29.0	9.5	0.3
$d(AG)_8$	1.39	4.1	1.3	0.07
$d(TC)_8$	0.81	27.5	9.0	0.2
dA_8	1.54	7.5	2.5	0.3
dC_8	0.74	5.2	1.7	0.04
dG_8	1.15	7.6	2.5	0.2
dT_8	0.87	12.0	3.9	0.06
none	—	3.05	1.0	—

* { [DNA] = 48 μM (base nucleotides)
[RecA] = 800 nM, [ATP] = 1 mM

(i) dA/dt is the average slope of at least two experiments at 340 nm.

(ii) k_{DNA} is the DNA dependent rate of ATP hydrolysis by RecA. c is a proportionality constant.

(iii) k_0 is the DNA independent rate of ATP hydrolysis by RecA.

(iv) $\pm\sigma$ is the confidence interval in R_{rel}, the relative ratio of the DNA dependent to the DNA independent rate of ATP hydrolysis by RecA.

ATPase than dC_{16}. The poly(dG) oligomers are both relatively poor cofactors with R_{rel} = 2.5 and this is apparently length independent. Interestingly, R_{rel} of the alternating pyrimidine-pyrimidine oligomer $d(TC)_8$ is seven times greater than R_{rel} of the alternating purine-purine oligomer $d(AG)_8$. Results for the eight homo-oligomers demonstrate the dependence of RecA ATPase activity on sequence composition of the ssDNA cofactors. The large differences found for the alternating sequence hexadecamers when dA_{16} and dT_{16} display comparable relative rates reveals that RecA ATPase activity is also affected by the nearest neighbor sequence of ssDNA cofactors.

DISCUSSION

Previous studies have shown that binding of RecA protein to long single strand DNA's several hundred to several thousand nucleotides in length is not sequence specific but binds with differing affinity depending on the sequence composition of the ssDNA (19,27,28). Rates of ATP hydrolysis by RecA in the presence of long ssDNA polymers also indicated a sequence dependence (19,28)

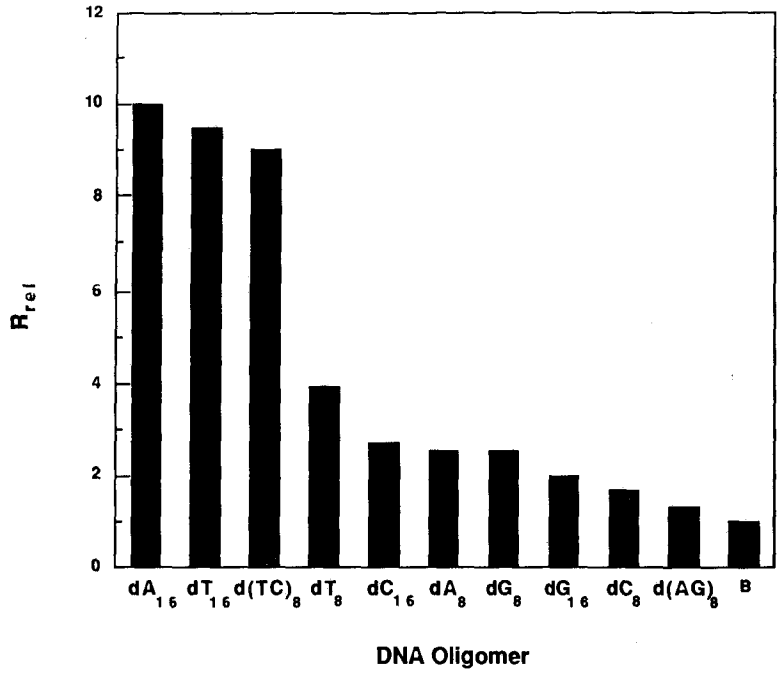

Figure I: R_{rel} for the various single strand DNA cofactors. R_{rel} is the ratio of the single strand DNA dependent rate of ATP hydrolysis by RecA protein to the DNA independent rate of RecA mediated ATP hydrolysis. B represents the blank with $R_{rel} = 1$.

However, a correlation between binding affinity of RecA to certain ssDNA sequences over others and the level of RecA ATPase activity was not found (27).

A recently published study investigated the binding of RecA to a series of heterogeneous sequence ssDNA oligomers ranging in length from 8 to 20 nucleotides (29). It was found that binding to ssDNA oligomers ($\leq$ 15 nucloetides) is considerably weaker than to long DNAs, and detection of the binding requires conditions where a large molar excess of RecA protein to ssDNA exists. Binding under any conditions is not even detectable for oligomers less than nine nucleotides in length (29). Although a sequence dependence of RecA binding to 15-mers was mentioned, protection of the ssDNA's from attack by DNAase or modification enzymes suggested that RecA binding to short ssDNA oligomers pimarily involves interactions with the phosphate backbone (29).

Not only is the binding affinity of RecA for ssDNA diminished with decreasing length of the DNA, oligomers less than 50 nucleotides are relatively poor cofactors for ATP hydrolysis (19,28). A ssDNA sequence and length dependence of RecA proteolytic activity was also reported (14,15) which, due to the supposed similarity of the modes of activation of RecA ATPase and proteolytic activity, implies a sequence dependence of RecA ATPase

activity. Results of our RecA ATPase assays directly verify this latter conjecture and demonstrate that although the overall level of activity is weaker, a dependence of RecA induced ATPase activity on the sequence of short ssDNA cofactors exists.

In the prevailing model of RecA/ssDNA binding, protein-protein interactions between RecA molecues along the ssDNA backbone are thought to be required for DNA binding and ATPase activity (30-32). The observed length dependence of RecA binding and ATPase activity indicates these interactions become stronger as the length of the ssDNA increases. If it is true, as has been previously suggested, that binding of RecA protein to ssDNA is a necessary but not sufficient requirement for ATPase activity (27), then the short DNA sequence dependence of ATPase activity reported here is consistent with this notion.

Detection of RecA protein ATPase activity under conditions where DNA binding is presumably weak, or not supported at all, might alternatively suggest that activation of lower levels of ATPase activity does not require binding of RecA with ssDNA and that sequence dependent conformational features of the ssDNA's are alone capable of activating the ATPase activity of RecA protein to relatively different levels. If this is the case, tight binding of RecA to ssDNA is not necessary for ATPase activation and the presence of ssDNA is sufficient for the sequence dependent activation of RecA ATPase activity.

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