

# Hydrolysis of Nucleoside Triphosphates Catalyzed by the *recA* Protein of *Escherichia coli*

HYDROLYSIS OF UTP\*

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Hydrolysis of UTP catalyzed by the *recA* protein of *Escherichia coli* is stimulated by both double- (DS) and single-stranded (SS) DNA. DS DNA-dependent UTPase activity has a sharp optimum near pH 6. SS DNA-dependent UTP hydrolysis also is optimal near pH 6, although considerable activity remains at pH 8. Both SS and DS DNA-dependent UTPase activities are non-linearly dependent on *recA* protein concentration at pH 6 but the SS DNA-dependent reaction shows a linear dependence on enzyme concentration at pH 8. The  $K_m$  for UTP in the SS DNA-dependent reaction decreases from 147  $\mu\text{M}$  at pH 8 to 33  $\mu\text{M}$  at pH 6. The  $K_m$  for UTP in the DS DNA-dependent reaction is 247  $\mu\text{M}$  at pH 6. In addition, the Hill coefficient for UTP in the SS DNA-dependent reaction decreases from 3.5 at pH 8 to 1.9 at pH 6, while in the DS DNA-dependent reaction, the Hill coefficient is 2.4 at pH 6. Thus, the UTPase activity of the *recA* protein differs from the ATPase activity of *recA* protein primarily in the pH dependence of  $K_m^{\text{UTP}}$ ,  $V_{\text{max}}$ , and response to enzyme concentration of the SS DNA-dependent hydrolysis reaction. These differences may be related to the inability of UTP to substitute effectively for ATP in *recA* protein-promoted annealing and assimilation of SS DNA.

The *recA* protein of *Escherichia coli* can promote the hybridization of complementary DNA sequences (either annealing of two single strands or assimilation of single strands into DS<sup>1</sup> DNA) (1-3) and the DNA-dependent hydrolysis of nucleoside triphosphates (4). Hybridization is specifically dependent on ATP (or dATP). However, both ATP (dATP) and UTP (dUTP) are hydrolyzed by the *recA* protein at a common or overlapping site on the enzyme (4). Both ATP( $\gamma\text{S}$ ) and UTP( $\gamma\text{S}$ ) stabilize *recA* protein-DNA complexes (5) and, thus, there is less nucleoside triphosphate specificity for the binding of *recA* protein to DNA than for its annealing activities.

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<sup>1</sup> The abbreviations used are: DS, double-stranded; ATP( $\gamma\text{S}$ ), adenosine 5'-O-(3-thiotriphosphate); UTP( $\gamma\text{S}$ ), uridine 5'-O-(3-thiotriphosphate); SS, single-stranded; BSA, bovine serum albumin; NTP, nucleoside triphosphate.

However, there are different effects of ATP and UTP on the structure of the *recA* protein (5).

Hydrolysis of ATP by *recA* protein is a complex reaction with different characteristics depending upon the DNA cofactor and pH (4, 6). Here we present a comparable characterization of UTP hydrolysis. The UTPase activity of *recA* protein is also affected by the DNA cofactor and pH and shows a complex dependence on substrate concentration. SS DNA-dependent UTP hydrolysis differs from ATP hydrolysis in that  $K_m^{\text{UTP}}$ ,  $V_{\text{max}}$ , and the dependence on *recA* protein concentration are all sensitive to pH. This finding suggests that one or more steps in the ATP hydrolytic cycle are ATP specific and possibly relate to the ability of ATP, but not UTP, to promote the hybridization reactions.

## EXPERIMENTAL PROCEDURES

All reagents and assays were as described in the previous paper (4). Initial velocities of UTP hydrolysis were determined by a time course unless otherwise noted.

## RESULTS

**Hydrolysis of UTP**—Hydrolysis of UTP was measured in the presence of SS DNA at pH 6.2 and pH > 7 and in the presence of DS DNA at pH 6.2. SS DNA-dependent UTP hydrolysis showed linear kinetics, but the extent was dependent on the initial UTP concentration (Fig. 1a). This effect was more pronounced at pH 8 than at pH 6.2 (data not shown). DS DNA-dependent UTP hydrolysis showed a brief lag before a linear rate was achieved (Fig. 1b) and also showed a concentration-dependent limitation in extent (Fig. 1c). These results contrast with ATP hydrolysis where the extent of hydrolysis in the presence of SS DNA is not limited by the initial ATP concentration and where the extent of DS DNA-dependent ATP hydrolysis is greater (4).

DNA-dependent UTPase activity co-purified with the ATPase activity of *recA* protein through phosphocellulose, DEAE-cellulose, and hydroxylapatite column chromatography (4). In view of this and other similarities with the ATPase activity, it is clear that the UTPase activity is intrinsic to the *recA* protein.

**Effect of pH on UTP Hydrolysis**—SS DNA-dependent UTPase activity showed a pH optimum near 6.5 but there was considerable activity at alkaline pH (Fig. 2a). This finding contrasts with ATP hydrolysis, which is independent of pH in the presence of SS DNA (4). DS DNA-dependent UTPase activity showed a sharper optimum than the corresponding ATPase activity at pH 6.2, with little activity above pH 7 (Fig. 2b). This behavior is similar to DS DNA-dependent ATPase activity (4) although the UTPase optimum appeared somewhat narrower. The optimum for SS DNA-dependent UTPase activity (pH 6.5) appeared to be slightly more alkaline than

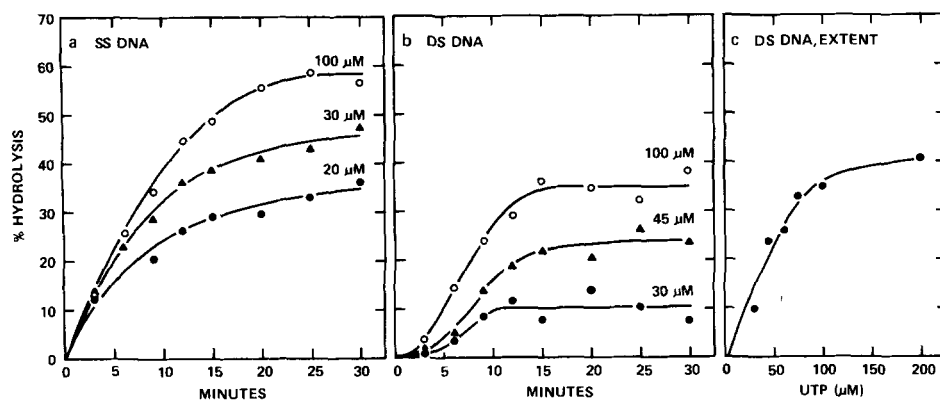


FIG. 1. Kinetics of UTP hydrolysis. Reactions (60  $\mu$ l) were performed in sodium maleate (pH 6.2) at 30  $^{\circ}$ C and contained either 84  $\mu$ M  $\phi$ X174 SS DNA and 0.88  $\mu$ M Fraction II (4) *recA* protein (a) or 77  $\mu$ M pZ6b DS DNA and 2.2  $\mu$ M Fraction II *recA* protein (b and c).

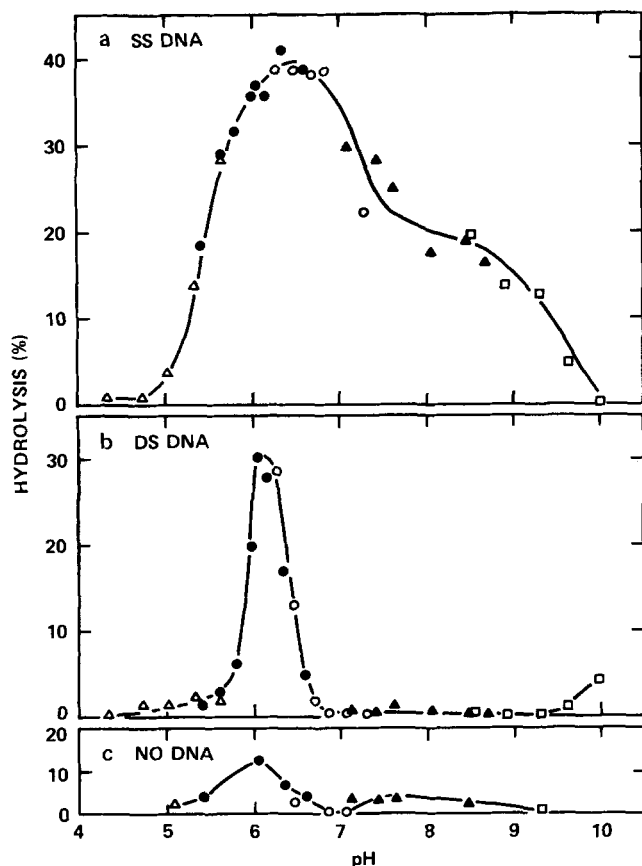


FIG. 2. pH dependence of UTP hydrolysis. Reactions (30  $\mu$ l) for measurement of SS DNA-dependent UTPase activity contained 20  $\mu$ M buffer, 10 mM  $MgCl_2$ , 1 mM dithiothreitol, 30  $\mu$ g/ml of BSA, 1.67 mM UTP, 74  $\mu$ M  $\phi$ X174 SS DNA, and 5.8  $\mu$ M Fraction II *recA* protein; incubation was for 20 min. Assays of DS DNA-dependent UTPase were the same except that 37  $\mu$ M P22 DS DNA was used, *recA* protein was 8.7  $\mu$ M, and incubation was for 30 min. In the DNA-independent UTPase assays (20  $\mu$ l), BSA was omitted, UTP was 50  $\mu$ M, and Fraction IV (4) *recA* protein was added to 6  $\mu$ M; incubation was for 60 min. pH was measured in 20 mM buffer in the presence of 10 mM  $MgCl_2$  at 25  $^{\circ}$ C.  $\Delta$ — $\Delta$ , Sodium acetate;  $\bullet$ — $\bullet$ , sodium maleate;  $\circ$ — $\circ$ , potassium phosphate;  $\blacktriangle$ — $\blacktriangle$ , Tris-HCl;  $\square$ — $\square$ , glycine-NaOH.

the DS DNA-dependent UTPase (pH 6.1).

UTP hydrolysis also occurred in the absence of DNA, although at a greatly reduced rate. This activity showed a pH profile that resembled the DNA-independent ATPase activity (4): an optimum near pH 6, a minimum near pH 7, and reduced activity above pH 7 (Fig. 2c).

**Effect of *recA* Protein Concentration on UTP Hydrolysis—**At pH 8, the turnover number (moles of UDP formed/min/mol of *recA* protein) for SS DNA-dependent UTP hydrolysis

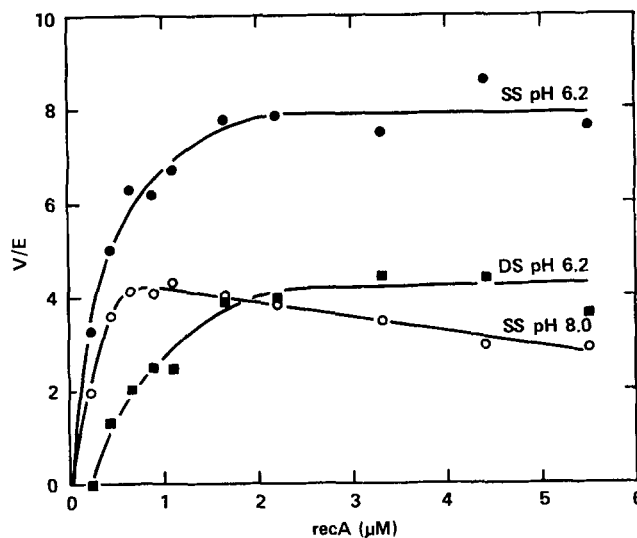


FIG. 3. Dependence of UTP hydrolysis on *recA* protein concentration. Reactions in Tris-HCl (pH 8.0) or sodium maleate (pH 6.2) contained 990  $\mu$ M UTP and either 84  $\mu$ M  $\phi$ X174 SS DNA or 103  $\mu$ M pZ6b DS DNA. *recA* protein was Fraction II.

was essentially constant from 0.4 to 2  $\mu$ M *recA* protein (Fig. 3), indicating that the velocity was proportional to *recA* protein concentration in a manner similar to the ATPase activity (4). As with ATPase activity, lower protein concentrations showed disproportionately reduced activity, possibly due to inactivation of *recA* protein or dissociation of *recA* protein oligomers (5, 7). At higher protein concentrations, activity also declined, possibly due to protein aggregation (5, 7) which is not observed at pH 6.2 (5).

At pH 6.2, the turnover number of both SS and DS DNA-dependent UTP hydrolysis depended on the protein concentration below 2  $\mu$ M *recA* protein, indicating an exponential dependence of velocity on enzyme concentration. The reaction with DS DNA was somewhat more dependent on protein concentration than the reaction with SS DNA. This finding contrasts with ATP hydrolysis, which is independent of protein concentration in the presence of SS DNA and is saturated at 0.8  $\mu$ M *recA* protein in the DS DNA-dependent ATPase reaction (4). These effects may be related to the dissociation of *recA* protein oligomers at pH 6.2 (5).

**Dependence of UTP Hydrolysis on UTP Concentration—**UTP hydrolysis showed a complex dependence on UTP concentration, affected by both the pH and the DNA cofactor (Fig. 4 and Table I). SS DNA-dependent UTP hydrolysis at pH 8 had a Hill coefficient of 3.5 at UTP concentrations below 100  $\mu$ M, while at pH 6.2, the Hill coefficient was reduced to 1.9. A similar effect was observed for ATP hydrolysis (6). However, unlike ATP hydrolysis,  $K_m^{UTP}$  also decreased, from 147  $\mu$ M at pH 8.0 to 33  $\mu$ M at pH 6.2. Furthermore, unlike ATP

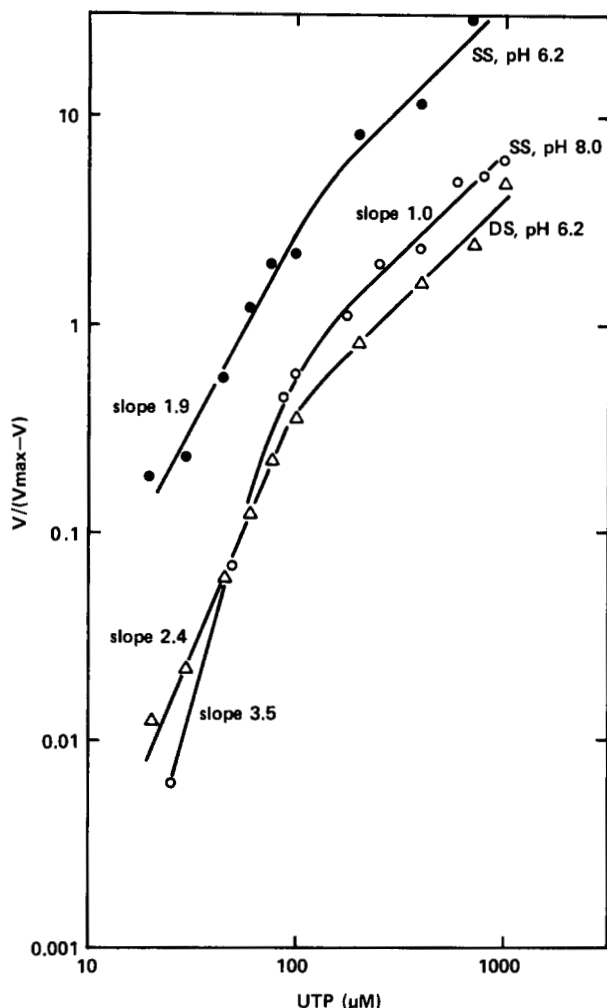


FIG. 4. Dependence of UTP hydrolysis on UTP concentration. Reactions were performed as in Fig. 1.  $V_{max}$  was determined from an Eadie-Hofstee plot.

TABLE I

Steady state kinetic parameters for UTP hydrolysis

Reactions were performed as described in Fig. 4.  $K_m^{UTP}$  and  $V_{max}$  were determined from an Eadie-Hofstee plot. The Hill coefficients are from Fig. 4.

DNA	pH	recA protein	$K_m^{UTP}$	$V_{max}/E$	Hill coefficient
			$\mu M$	$mol\ UDP/min/mol\ recA\ protein$	
SS	8.0	0.88	147	5.7	3.5
SS	6.2	0.88	33	8.2	1.9
DS	6.2	2.2	247	6.3	2.4

hydrolysis, the  $V_{max}$  for SS DNA-dependent UTP hydrolysis was also pH dependent (Figs. 2 and 3 and Table I), being greatest at pH 6.2. Thus, SS DNA-dependent UTP hydrolysis may have different rate-limiting steps at pH 6.2 and 8.0.

DS DNA-dependent UTP hydrolysis by *recA* protein had a Hill coefficient of 2.4 (below 100  $\mu M$  UTP) and a  $K_m^{UTP}$  of 247  $\mu M$ . Thus, like the ATPase reaction, the  $K_m^{UTP}$  was higher with DS than SS DNA.

**Inhibition of UTP Hydrolysis by ATP**—All of the hydrolytic reactions catalyzed by *recA* protein show the same nucleotide specificity (4), and hydrolysis of ATP is competitively inhibited by UTP (6). UTP hydrolysis was also competitively inhibited by ATP (data not shown). The  $K_i^{ATP}$  was approximately 23  $\mu M$ , close to the  $K_m^{ATP}$  for ATP hydrolysis (6).

**Polynucleotide Requirement for UTP Hydrolysis**—UTP

hydrolysis was stimulated equally well by circular ( $\phi X174$ ) or linear (calf thymus) SS DNA as well as by  $(dT)_{300}$ . Thus, there is no relationship between the base sequence of the DNA cofactor and specificity for the nucleoside triphosphate substrate.

#### DISCUSSION

UTP hydrolysis by the *recA* protein shows three important differences from ATP hydrolysis. (i) SS DNA-dependent UTP hydrolysis has a pH optimum at pH 6.2 as compared to the broad pH optimum ranging from pH 6 to 9 for ATP hydrolysis. (ii) In contrast to SS DNA-dependent ATP hydrolysis, SS DNA-dependent UTP hydrolysis shows a nonlinear dependence on enzyme concentration at pH 6.2. (iii)  $K_m^{UTP}$  with SS DNA is lower at pH 6.2 than at alkaline pH, whereas  $K_m^{ATP}$  shows no pH dependence.

As described previously (4), we view the ATP hydrolytic cycle as a sequence in which the binding of ATP and SS DNA changes the conformation of *recA* protein, activating it for hydrolysis. At pH 6.2, the conformation of the enzyme changes spontaneously and certain steps which are ATP-dependent are bypassed. This leads to a reduction in the Hill coefficient for ATP (6). The similarities between the UTPase and ATPase activities indicate that some steps in this sequence are shared. However, the finding that both  $V_{max}$  and  $K_m^{UTP}$  are affected by pH indicates that UTP does not substitute efficiently for ATP in those steps that are bypassed at pH 6.2. Thus, at alkaline pH, ATP, but not UTP, efficiently induces the conformational states required for hydrolysis while at acid pH, the NTP requirement is obviated. Hence  $V_{max}$  and  $K_m$  are independent of pH in the ATPase reactions, but are affected by the low pH bypass in the UTPase reaction.

The nonlinear dependence of UTP hydrolysis on enzyme concentration at pH 6.2 demonstrates that oligomerization is likely to be important in these reactions. This follows from the observations that at pH 6.2, the *recA* protein exists in a lower oligomeric form than at pH 7.5, and this low pH form oligomerizes in the presence of ATP but not UTP (5). Since UTP is also inefficient in promoting the single strand annealing and assimilation reactions, this oligomerization may be an important part of these reactions, for instance in the pairing of DNA chains.

These and other observations demonstrate that UTP interacts efficiently with the *recA* protein of *E. coli*. Hydrolysis of UTP is comparable to ATP, and ATP and UTP have similar effects on the binding of *recA* protein to DNA (5). However, the weak stimulation of SS DNA annealing (1), assimilation (2, 3), and protease (8) activities of *recA* protein by UTP indicates that, *in vivo*, UTP must function in a manner different from ATP. Whether the role of UTP is as a regulator of ATP-dependent reactions or as a cofactor for additional activities of the *recA* protein remains to be clarified.

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