Selective Inhibition of the DNase Activity of the recBC Enzyme by the DNA Binding Protein from *Escherichia coli*

(Received for publication, November 11, 1975)

Vivian Mack† and Stuart Linn

From the Department of Biochemistry, University of California, Berkeley, California 94720

In the presence of the *Escherichia coli* DNA binding protein, single-stranded DNA is resistant to both the endo- and exonucleolytic activities of the recBC DNase. Linear duplex DNA, on the other hand, is unwound at a normal rate, but converted to large, single-stranded fragments which are resistant to further hydrolysis. Therefore, in the presence of the binding protein and linear duplex DNA, the recBC enzyme acts not as a DNase, but primarily as an ATP-dependent unwinding enzyme, able to generate large, single-stranded material. Duplex circular DNA containing short, single-stranded gaps is also resistant to the hydrolysis in the presence of the binding protein.

The recBC enzyme (exonuclease V) of *Escherichia coli* is an ATP-dependent DNase with a variety of *in vitro* catalytic properties (1, 2). It is a potent ATPase requiring a polydeoxyribonucleotide cofactor, and it is an ATP-dependent exonuclease on both duplex and single-stranded DNA. It can function as an ATP-stimulated endonuclease on single-stranded, but not on duplex circular DNA, even if the latter has been nicked, ultraviolet-irradiated, or X-irradiated. However, duplex circular DNA containing single-stranded gaps of approximately five nucleotides can be degraded (3).

Gaps in the DNA are proposed to exist transiently in the cell after the excision of ultraviolet-induced thymine dimers (4), perhaps during replication (5), and as part of the recombination process (6). What prevents the recBC DNase from degrading such DNA *in vivo*? The activity of the DNase appears to be at least in part by the product of the recA gene, since recA mutants exhibit both spontaneous and ultraviolet-induced DNA breakdown caused to a large extent by the recBC DNase (7, 8). However, the recA product has not been isolated or identified as yet. Therefore, we have initiated a study of interactions of the DNase with other purified cellular proteins in an attempt to elucidate the nature of the enzyme. In this report, we describe the effects of the *E. coli* DNA binding protein (9–11) on the *in vivo* activities of the recBC enzyme. Hendler et al. (12) recently described a catalytic interaction between recBC enzyme and the *E. coli* DNA polymerases.

**EXPERIMENTAL PROCEDURE**

Two independent preparations of apparently homogeneous DNA binding protein from *Escherichia coli* were employed; they were provided by I. Molineux of Massachusetts Institute of Technology (10) and J. Weiner of Stanford University (11). We have not detected any significant differences in the properties or specific activity of the two preparations and, therefore, will not distinguish between them. The glycerol gradient fraction of the recBC DNase was used throughout; it was purified and assayed upon linear DNA as described previously (1).

The following [H]DNAs (5,000 to 10,000 cpm/nmol) were prepared: *E. coli* DNA by the method of Lehman (14), phage T7 DNA by the procedure of Richardson (15), fd single-stranded, circular phage DNA and closed, double-stranded circular replicative form DNA (RFI), as described previously (16). DNA quantities are expressed in nanomoles of nucleotide.

**RESULTS**

**Single-stranded DNA Substrates**—Under conditions which maximize DNase activity (standard assay conditions), binding protein caused progressively greater inhibition as its concentration was increased in either an endonuclease assay with circular fd DNA or an exonuclease assay with denatured *Escherichia coli* DNA (Table I). A level of 2.3 μg of binding protein/nmol of nucleotide is sufficient to saturate phage φX174 DNA (11), and concentrations greater than this value are clearly sufficient essentially to inhibit completely the hydrolysis of the single-stranded substrate by the recBC enzyme.

**Linear Duplex Substrates**—Because the binding protein exhibits little affinity for double-stranded DNA under our assay conditions (9), we expected that the duplex exonuclease activity of the recBC enzyme would be affected only slightly by binding protein, if at all. However, at subsaturating binding protein concentrations the activity of the DNase, as measured by release of acid-soluble material, actually was stimulated (Table I). Partial inhibition was observed only when binding protein was added at levels in excess of that required to saturate an equivalent amount of single-stranded DNA.

In an earlier report (17), we presented evidence that degradation of duplex DNA by the recBC enzyme takes place through

---

*This research was supported by Contract AT(04-3)-34 from United States Energy Research and Development Administration and Grant GM18920 from the National Institutes of Health.
† Postdoctoral Fellow of the American Cancer Society (Grant PF052). Present address, Waksman Institute of Microbiology, Rutgers University, New Brunswick, New Jersey 08903.
the formation of large single-stranded intermediates which are subsequently hydrolyzed by the single strand exonuclease activity to acid-soluble products. The intermediates could be most readily detected and isolated from brief reactions that contained high concentrations of ATP (5 mM) and NaCl (100 mM). These conditions might be analogous to the in vivo environment and appear to favor the unwinding of the DNA strands while retarding their degradation by the enzyme. Under these conditions the release of acid-soluble material from duplex DNA was more strongly inhibited than under standard reactions (see Fig. 1) and single-stranded DNA was still fully protected by the binding protein.

Of course, the absence of acid-soluble material is not an indication of whether the larger, single-stranded products were formed. Such products can, however, be detected by sedimentation through sucrose gradients. T7 duplex DNA was thus incubated with recBC enzyme and binding protein under standard assay conditions as well as with 5 mM ATP plus 100 mM NaCl, then split into 2 aliquots. One portion was used to determine acid-soluble radioactivity and the other was sedimented through a sucrose gradient. The gradient profiles (Fig. 1) imply that enzymatic degradation of intact T7 DNA to intermediate sized products is not inhibited by DNA-binding protein, but that the further degradation of these products is inhibited, particularly in high ATP-NaCl reactions. It was previously determined that the material in the large peak near the top of the gradient is 50 to 100% single-stranded with a size range of 135 to 1400 nucleotides (17). From the above experiments it appears that the DNA binding protein does not affect the formation of partially and wholly single-stranded intermediates from duplex DNA, but that it does prevent the degradation of these to acid-soluble limit products.

As an alternative verification of this hypothesis, we measured directly the influence of binding protein on the generation of single-stranded material from duplex DNA. Sensitivity to digestion by the single-stranded DNA-specific enzyme, *E. coli* exonuclease I (14), was used to quantitate the formation of single-stranded DNA. Under both standard reaction conditions and high ATP-NaCl conditions, the addition of DNA binding protein substantially inhibited the formation of acid-soluble but not single-stranded DNA (Table II).

It thus may be concluded that the binding protein does not strongly inhibit the generation of single-stranded material by the ATPase-mediated recBC enzyme unwinding reaction, but instead inhibits the degradation of this material to acid-soluble oligonucleotides. This observation is analogous to the selective inhibition of the DNase activity of phage T4 DNA polymerase by the T4 binding (gene 32) protein (18). It is clear that binding protein and enzyme must compete for the single-stranded DNA as it is formed, and thus at low levels of the former, inhibition is not observed (Table I). The explanation of the
Inhibition of recBC DNase by E. coli DNA Binding Protein

Free duplex termini do not likely occur in the cell, and the results presented above suggest that gaps in duplex DNA (or other single-stranded DNA) might be protected by the binding protein. The protection of single-stranded DNA in vivo was in fact observed by Benzinger et al. (19) who examined the transfection of rec+ and rec− spheroplasts with various viral DNAs to try to determine the specificity of the recBC DNase in vivo. In this way they showed that the single-stranded endonuclease and exonuclease activities did not appear to be significant; only linear duplex DNAs had their infectivity substantially reduced by the recBC DNase. In fact the short, single-stranded termini of λ DNA were sufficient to preserve infectivity in cells containing the recBC DNase. This in vivo specificity is that noted for the enzyme in vitro in the presence of the binding protein.

Future studies concerning the in vivo function of the enzyme and its possible role in recombination and replication might thus to consider positive as well as negative control factors and, perhaps, novel substrates, cofactors, and reactions. A notable example in this regard is the isolation by Hendler et al. (12) of complexes of recBC enzyme with each of the Escherichia coli DNA polymerases, I, II, and III. The complexes are novel in that they promote ATP-stimulated DNA synthesis in a reaction preferring duplex DNA. Regarding recombination, the concept of the generation of single-stranded DNA for the initiation of the recombination pathway through "single-strand aggression" has recently been emphasized (20, 21). We might then hypothesize that the major contribution of the recBC enzyme to recombination would be the unwinding of DNA as observed in the presence of binding protein. The DNase activity might be a secondary function—the restriction of foreign DNA (22), or perhaps the termination of a recombination region by the placement of a judicious nick in the recombining DNA molecules. Any rate the ability of the enzyme to continue to unwind DNA while its DNase activity has been suppressed (whether brought about by binding protein or some other unknown factor) is unique, and ought to be taken account of in consideration of its in vivo function. It certainly suggests an alternative reaction for the enzyme, other than the degradation of DNA to nonfunctional oligonucleotides.

The effect of the E. coli DNA binding protein upon many other nucleases has recently been described (23). It was shown that the protein inhibited pancreatic DNase, venom phosphodiesterase, the Neurospora crassa and Aspergillus S1 single-stranded DNA-specific DNases, and the nucleases associated with E. coli DNA polymerases I and III and phage T4 DNA polymerase. On the other hand, there was little or no inhibition of micrococcal nuclease, or the nucleases associated with E. coli DNA polymerase II and phage T7 DNA polymerase. E. coli exonuclease I was stimulated by the protein and the latter three enzymes were shown to form direct complexes with the protein in the absence of DNA. In view of these results the inhibition reported here of the DNase activity of the recBC enzyme is not unique, or even surprising. What we feel is important, however, is that the enzyme still maintains its DNA-dependent ATPase activity, and that with duplex DNA as a cofactor.

The experiments described in this report indicate that an appropriate question is not only what elements keep the recBC enzyme under control, but what elements allow it to function.

Table II

<table>
<thead>
<tr>
<th>Reaction conditions</th>
<th>Binding protein</th>
<th>recBC enzyme</th>
<th>DNA rendered acd-soluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>+</td>
<td>−</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>0.006</td>
</tr>
<tr>
<td>High ATP</td>
<td>+</td>
<td>+</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>0.073</td>
</tr>
</tbody>
</table>

stimulation that is instead observed in this case is ambiguous: either low levels of the protein stimulate the unwinding and thus the DNase indirectly, or the DNase is stimulated directly. The former alternative might be more likely as we have observed that in the presence of 2.1 µg of binding protein/nmol of DNA, ATPase is stimulated by 60% with duplex DNA present, but inhibited by 60% with single-stranded DNA as a cofactor.

Gapped Circular Duplex DNA Substrate—The recBC enzyme hydrolyzes duplex circular fd replicative form DNA provided that the DNA contains a single-stranded gap of at least roughly five nucleotides (3). Under standard assay conditions binding protein inhibited this reaction, with standard reaction conditions or high ATP·NaCl conditions.

The absence of activity on this substrate in the presence of binding protein might be taken to indicate that recBC enzyme normally initiates its action by endonucleolytic cleavage at the single-stranded DNA gap, not by unwinding of the duplex from a gap terminus. In the latter case we might not have expected the binding protein to inhibit completely, but instead to have given results such as those found for linear duplex DNA (Table I). In support of this idea we also have observed that the ATPase activity of the enzyme with gapped DNA is similarly inhibited by binding protein. However, the binding protein might merely act by making single-stranded DNA and therefore, gaps, unrecognizable to the enzyme. For this reason, the mode of degradation from gaps remains unknown.¹

¹The question of how the enzyme acts from gaps is important, as it has obvious implications regarding how the enzyme might take part in genetic recombination. In the absence of ATP, where only the single-stranded DNA-specific endonuclease is active, we observed that gapped circles are resistant to large excesses of recBC enzyme. These results could imply that in fact unwinding is the key step in initiation of degradation from gaps. However, we have been unsuccessful in demonstrating such unwinding without concurrent hydrolysis of the entire molecule, even with binding protein present. Further exploration of this problem is clearly warranted.

DISCUSSION

The experiments described in this report indicate that an appropriate question is not only what elements keep the recBC enzyme under control, but what elements allow it to function.

¹The question of how the enzyme acts from gaps is important, as it has obvious implications regarding how the enzyme might take part in genetic recombination. In the absence of ATP, where only the single-stranded DNA-specific endonuclease is active, we observed that gapped circles are resistant to large excesses of recBC enzyme. These results could imply that in fact unwinding is the key step in initiation of degradation from gaps. However, we have been unsuccessful in demonstrating such unwinding without concurrent hydrolysis of the entire molecule, even with binding protein present. Further exploration of this problem is clearly warranted.
Acknowledgments—We are grateful to Drs. Ian Molineux and Joel Weiner whose provision of binding protein made these studies possible.

REFERENCES
Selective inhibition of the dnase activity of the recBC enzyme by the DNA binding protein from Escherichia coli.

V Mackay and S Linn


Access the most updated version of this article at [http://www.jbc.org/content/251/12/3716](http://www.jbc.org/content/251/12/3716)

Alerts:
- When this article is cited
- When a correction for this article is posted

[Click here](http://www.jbc.org/content/251/12/3716.full.html#ref-list-1) to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/251/12/3716.full.html#ref-list-1](http://www.jbc.org/content/251/12/3716.full.html#ref-list-1)