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Inactivation of the ATP-dependent DNase of *Escherichia coli* After Infection with Double-Stranded DNA Phages

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The ATP-dependent DNase activity of *Escherichia coli* disappeared or was markedly reduced after infection with double-stranded DNA phages, T2, T3, T4, T5, T6, T7, λ, φ80, and P1, but not with the single-stranded DNA phage f1, or the RNA phage Qβ. This DNase activity was not reduced when chloramphenicol was added prior to phage infection.

Recent experiments have shown that the ATP-dependent DNase, which is the product of the *recB* and *recC* genes of *E. coli* (1, 2, 5, 8, 12), is inactivated after infection of bacteriophage λ or T4 (11, 13, 15). In the case of λ, the phage produces the inhibitor of this DNase which is the protein product of the phage gene, *gam* (9). The inhibitor (the *gam* protein) is highly specific for the ATP-dependent DNase and probably forms a protein-protein complex with it (9; A. Karu, Y. Sakaki, H. Echols, and S. Linn, The Gatlinburg Symposium, Mechanisms in Recombination, in press). The *gam* protein is necessary for the development of λred^- in a $recA^-$ host, apparently because certain forms of replicating λ DNA are susceptible to this DNase (4). Although only the *gam* protein of phage λ has been studied extensively, other phages may produce a "*gam*-like" protein to inactivate the ATP-dependent DNase, since this DNase is thought to be destructive to phage DNA and possible intermediates of DNA replication (3, 4, 10). For further understanding of the role of the *gam* or "*gam*-like" protein in bacteriophage development, I have investigated the effect of phage infection on the ATP-dependent DNase activity. In this report, I will show that the ATP-dependent DNase activity disappears or is markedly reduced in the cells infected with the double-stranded DNA phages, T2, T3, T4, T5, T6, T7, λ, φ80, and P1.

After infection at a multiplicity of 5, phages were grown at 37 C for 15 min in Tryptone broth (1% Tryptone [Difco], 0.5% NaCl). Then, the cells were washed, resuspended at 5×10^9 cells/ml in 10 mM Tris, pH 8.0, 10 mM $MgCl_2$, 10 mM β-mercaptoethanol, and sonicated for 1 min with an Arteck Sonic Dismembrator at position "50." Cell debris was removed by centrifugation and the ATP-dependent double-stranded exonuclease activity in the supernatant fraction was measured by the method of Unger and Clark (13). As summarized in Table 1, this DNase activity was markedly reduced or undetectable in the extracts from the cells infected with double-stranded DNA phages (T2, T3, T4, T5, T6, T7, λ, φ80, and P1). The disappearance of this DNase activity was not observed when chloramphenicol was added prior to phage infection (Table 1). Therefore, it was concluded that this disappearance of this DNase activity is caused by phage coded protein(s). On the other hand, the infections of single-stranded DNA phage f1 and RNA phage Qβ had no effect on this DNase activity (Table 1). In the T7-infected cell extract, the DNase activity was also measured at pH 7.0, 8.0, 9.0, and 9.5, and at 60, 330, and 1,000 μM ATP. In no case was ATP-dependent DNase activity detected. Also, the ATP-dependent single-stranded exonuclease activity of this DNase (6) was not detected in the extract (data not shown). Although only the T7-infected cell extract was studied under each set of conditions, these results make it unlikely that this apparent loss of the ATP-dependent DNase activity is due to an artifact of crude extract assay such as the consumption of ATP by phage coded ATPase.

It is interesting that the ATP-dependent DNase is inactivated after infection by all the double-stranded DNA phages tested here. The inactivation of this DNase might be generally important for the development of all double-stranded DNA phages. For example, T7 is known to replicate as a linear molecule (14). The inactivation of this DNase might be necessary for T7 to protect its DNA, since this DNase is thought to be very destructive to noncircular native DNA (2, 6, 10). Further studies are, however, required to understand the biological role of the inactivation of the ATP-dependent

TABLE 1. *Effect of phage infection on the ATP-dependent DNase activity*

E. coli K12 strain	Infecting phage[a]	ATP-dependent DNase act (units/mg of protein)[b]	
		− Chloramphenicol	+ Chloramphenicol[c]
C600	None	8.46	7.86
C600	T2	0.49	9.25
C600	T3	ND[d]	6.22
C600	T4D	ND	7.11
C600	T6	ND	7.11
C600	T7	ND	6.88
C600	λ	ND	Not tested
C600	P1	2.06	Not tested
Ymel(F^+)	None	6.95	6.96
Ymel(F^+)	ϕ80	ND	5.93
Ymel(F^+)	f1	6.10	Not tested
Ymel(F^+)	Qβ	5.40	Not tested
Ymel(F^+)	None	7.83	6.75
Ymel(F^+)	T5	0.48	5.80

[a] Phage infection was done at 25 C for 10 min in peptone broth (1% peptone, 0.1% glucose, 50 mM NaCl, 0.1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.32 mM KH_2PO_4) for T phages, in 10 mM Tris, pH 7.5, 10 mM $MgCl_2$ for λ, in 10 mM Tris, pH 7.5, 1 mM $MgCl_2$, 1 mM $CaCl_2$ for ϕ80, f1 and Qβ, and in L-broth (1% peptone, 0.5% yeast extract, 0.1% glucose, 0.5% NaCl, 1 mM $CaCl_2$) for P1. All phages used here were identified as follows. T2, morphology under electron microscope and less sensitivity to T4 antiserum. T3, morphology under electron microscope T4, sensitivity to T4 antiserum. T5, T6, inability to plate on each resistant host. T7, inability to plate on F^+ host. ϕ80, λ, inability to plate on each lysogen. f1, *Qβ*, inability to plate on F^- host. P1, transducing activity.

[b] One unit of this DNase renders 1 nmol of DNA-nucleotide acid soluble in the assay condition. Protein was determined by the method of Lowry et al. (7).

[c] Chloramphenicol was added to the culture to a concentration of 60 μg/ml 5 min prior to infection.

[d] ND, Not detectable.

DNase during bacteriophage development. In addition, the biochemical mechanism of the inactivation of this DNase is unknown. Attempts to study the inhibitory activity in vitro have been unsucessful so far, except in the case of λ (9).

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