INHIBITION OF EXONUCLEASE V AFTER INFECTION OF E.COLI BY BACTERIOPHAGE T7

W. Wackernagel and U. Hermanns

Ruhr-Universität, Abteilung Biologie, Lehrstuhl für Biologie der Mikroorganismen, 4630 Bochum, W-Germany

Received August 2,1974

SUMMARY

Exonuclease V (recBC DNase) is inactivated in E.coli between 4 and 7 min after infection by T7. This process requires protein sythesis. The inactivation does not occur when T7 is deficient for its RNA polymerase and thus does not express the genes involved in DNA replication and phage maturation. Some implications of this new function of T7 are discussed with respect to the processes of infection and DNA replication.

INTRODUCTION

The destruction of the biological activity of linear double-stranded DNA entering cells of E.coli has been attributed to the action of the ATP-dependent exonuclease V hereafter called exo V (recB recC-DNase; 1, 2, 3) by two lines of experiments. First, transfection of spheroplasts by various phage DNA was found to be about 100 fold more effective in strains lacking exo V, as observed with T4 DNA (4), inverted λ DNA (5) and T7 DNA (Wackernagel and Sriprakash, unpublished results). Second, experiments on the genetic transformation of E.coli were only successful with strains which were defective for exo V but had regained recombination-proficiency by secondary mutations (6, 7).

By contrast, the DNA of phage T7 has been shown to replicate as a linear duplex at least until after the first round of replication (8, 9) in normal, i.e. exoV$^+$ strains of E.coli. Such an intermediate would be a good substrate for the deleterious activity of exo V. Consequently, one might predict a protective function coded for by the T7 genome that antagonizes exo V

activity after infection. This paper reports the observation that in cells infected by T7 the activity of exo V decreases rapidly by a process that requires protein sythesis.

MATERIALS AND METHODS

Bacterial and phage strains. Exo V$^+$ strains of E.coli K12 were AB1157, W3550 (su$^-$) and C600 (su$^+$) (10). JC7620 recB21 recC22 was an exoV$^-$ strain (11). T7 wildtype, T7 am 94 (12) and the deletion mutant T7 H3 (13) were generously provided by Drs. W. Rüger, P. Herrlich and W.C. Summers, resp.

Preparations of extracts from infected cells. A modification of the procedure developed by Godson and Sinsheimer (14) was used. Cells were aerated at 30° to a density of about $2 \cdot 10^8$/ml in TBY-medium (1% tryptone, 0.5% yeast-extract, 0.5% NaCl). Phages were added at a multiplicity of about 10 and incubation continued. At various times samples of 15ml were withdrawn and quickly chilled in ice. Na-azide was added to a final concentration of 0.02M. The cells were washed in an ice cold solution of 0.85% NaCl and resuspended in 0.18ml of a solution containing 0.01M Tris-HCl, pH 8.0, 0.05M Na-azide and 20% sucrose. The mixture was placed on ice and 0.01ml of lysozyme (1mg/ml in 0.01M Tris-HCl, pH 8.0) and 0.005ml Na_3-EDTA (0.1M) were added. Two min later 0.185ml of 0.1M $MgSO_4$ and 0.075ml of 5% Brij-58 in 0.01M Tris-HCl, pH 8.0, were added. The suspension was incubated for 5 min at 37° followed by 1 h at 4°. After centrifugation for 10 min at 15000g the supernatant was collected and stored in ice until assay of enzyme. Protein concentrations in the extracts were determined by the method of Lowry et al. (15).

Exo V assay. The procedure used was adapted from that described by Barbour and Clark (3). The substrate was ^{3}H thy-

midine labeled DNA from phage P22 with a specific activity of $3.9 \cdot 10^6$ cpm/μM nucleotide. The reaction mixture contained in a total volume of 0.5ml 5 μmoles Tris-HCl, pH 8.0, 0.1 μmoles ATP, 10 nmoles ^{3}H-DNA and 0.04ml of cell extract. The mixture was incubated for 30 min at 37°, then chilled in ice followed by the addition of 0.5ml of 10% trichloracetic acid and 0.2ml of carrier DNA (2.5mg/ml). After 10 min on ice precipitated nucleic acids were sedimented for 10 min at 12000g and the radioactivity in the supernatant was determined in a Packard scintillation counter. The scintillation mixture consisted of toluene containing 36% Triton X-100 and 3.2g PPO plus 64mg POPOP per 1000ml. A parallel assay was performed in which ATP was omitted from the reaction mixture. The difference in the amount of acid soluble radioactivity produced in the two assays was the basis for the calculation of exo V activity. One unit of enzyme produces 1 nmole of 5% TCA soluble nucleotides at 37° in 30 min. Specific activities are given as units of activity per mg of protein.

RESULTS

Exo V possesses four enzymatic activities (16, 17). We measured only one of them, namely the ATP-dependent exonuclease activity on duplex DNA as an assay for the presence of exo V in crude cell extracts. This activity decreased sharply 4 min after infection of E.coli wildtype cells by phage T7 and was undetectable 3 min later (Fig. 1). Simultaneously with the decrease of exo V activity there appeared ATP-independent DNase activity which can be attributed to T7-specific enzymes, such as the gene 6-exonuclease (18) and the exonuclease associated with the gene 5-DNA polymerase (19, 18). This ATP independent

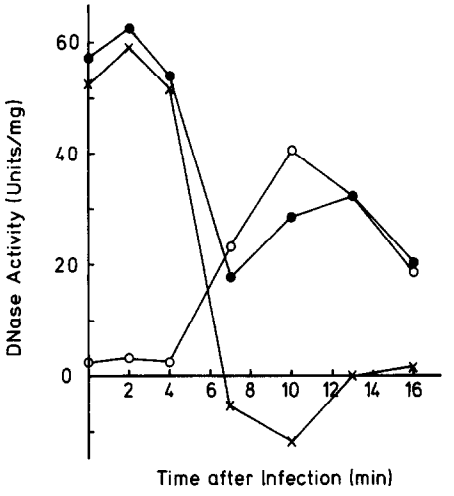

Fig. 1 The inactivation of exo V in T7 infected E.coli. Cells of strain AB1157 were infected at 30° by phage T7 wild-type and DNase activities were determined at the times indicated with linear duplex DNA as a substrate. The procedures are described in Materials and Methods. o ATP-independent DNase (a); ● ATP-independent DNase plus exo V (b); X exo V (b-a)

DNase activity was also observed in T7 infected cells of E.coli recB-C- (JC7620), which lack exo V (11). When wildtype cells were infected in the presence of chloramphenicol the level of exo V remained constant for at least 16 min (Fig. 2a). This indicates that exo V activity is stable as long as protein synthesis is inhibited in the infected cells. As expected, also no T7 DNases were produced under these conditions. We conclude that a T7 specific protein which we will call ϵ-protein is involved in the inhibition of exo V.

Because of the linearity of T7 DNA a protection against exo V might be required immediately after phage infection. Therefore it was expected that the gene for ϵ-protein was among the early genes (coding for the class I proteins) transcribed by E.coli RNA polymerase (20). Among these early functions the protein kinase (21) appeared to be a likely candidate, but normal inactivation of exo V was observed with a H3 deletion mu-

tant of T7 (13) which is defective for protein kinase (21). However, no activation of exo V occured after infection of a su⁻ strain (W3550) by a gene 1-amber mutant of T7 (Fig. 2b). Gene 1 codes for the T7 RNA polymerase (a class I protein) which is required for the transcription of genes 1.7 to 19. From these genes the class II and III proteins are synthesized with a small time lag relative to the class I proteins (20). Thus the ε-protein is not among the very first proteins synthesized in the process of T7 development. This conclusion will be discussed below in more detail. In preliminary experiments inactivation of exo V was observed with amber mutants of T7 in genes 2, 3, 4, and 6 (20).

DISCUSSION

Although as yet the T7 gene responsible for the inactivation of exo V has not been identified its function might be a stringent requirement for the unimpaired replication of T7 DNA, since the intermediates of replication which are linear DNA

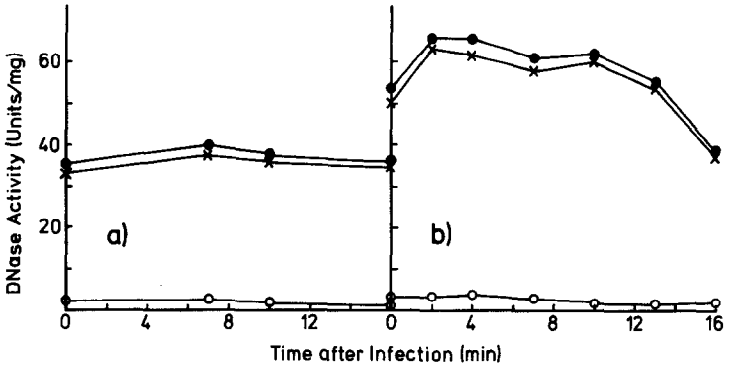

Fig. 2 a) Exo V activity in E.coli infected by T7 in the presence of chloramphenicol. Cells of strain AB1157 were infected by T7 wildtype. Chloramphenicol (100 ug/ml) was added 5 min prior to infection.
b) Exo V activity in E.coli W3550 (su⁻) infected with T7 am 28 (gene 1 mutant).
For symbols and other details see legend to Fig. 1.

structures at early (8, 9) and presumably late stages of replication (22) would be sensitive to the destructive action of exo V. This notion is supported by the observation that the inactivation of exo V is complete before the start of DNA replication which occurs about 10 min after infection at 30° (20).

A similar function as reported here for T7 has recently been found in phage λ . The gamma-protein specified by the gam gene (23) has been identified as the direct inhibitor of exo V (24, 25). The role of the gamma protein might be the protection of late intermediates in λ replication which might involve linear structures (26). Inactivation of exo V after infection by phage T4 has also been reported (27).

What is the reason for the extensive inactivation of T7 DNA by exo V after transfection of spheroplasts as compared with the stability of T7 DNA after normal infection? From several possible explanations we will present two: (i) After normal infection both ends of the DNA duplex are temporaryly protected by association with some cellular component, e.g. a membrane constituent. The release is caused by replication when exo V is already inactivated. (ii) The infecting DNA enters an cellular compartment free of exo V where it stays during the early stages of intracellular phage development. In transfection the DNA presumably enters the cell at a site different from the point predetermined by the phage receptor and thereby fails mostly to establish its exo V-protected state before attack by the enzyme.

Since the inhibition of exo V requires protein synthesis we have postulated the involvement of a T7 specified protein in this process, the ε-protein. In an in vitro assay with crude cell extracts the heat stability of the inhibitor has been de-

termined (manuscript in prep.). The results provided evidence for the protein character of the inhibitor. Its purification is in progress. The in vitro properties of this protein will show whether it is the direct inhibitor of exo V. To answer the question whether the inhibitor and ε-protein are identical will require the identification of the inhibitor as a T7 specified protein.

REFERENCES

1. Wright, M. and Buttin, G., Bull.Soc.Chim.Biol. 51, 1373 (1969)
2. Oishi, M., Proc.Natl.Acad.Sci.USA 64, 1292 (1969)
3. Barbour, S.D. and Clark, A.J., Proc.Natl.Acad.Sci.USA 65, 955 (1970)
4. Wackernagel, W., Virology 48, 94 (1972)
5. Wackernagel, W. and Radding, C.M., Virology 52, 425 (1973)
6. Wackernagel, W., Biochem.Biophys.Res.Comm. 51, 306 (1973)
7. Cosloy, S.D. and Oishi, M., Proc.Natl.Acad.Sci.USA 70, 84 (1973)
8. Wolfson, J., Dressler, D. and Magazin, M., Proc.Natl.Acad. Sci.USA 69, 499 (1972)
9. Dressler, D., Wolfson, J.and Magazin, M., Proc.Natl.Acad. Sci.USA 69, 998 (1972)
10. Bachmann, B.J., Bacteriol.Rev. 36, 525 (1972)
11. Barbour, S.D., Nagaishi, H., Templin, A., and Clark, A.J., Proc.Natl.Acad.Sci.USA 67, 128 (1970)
12. Studier, F.W., Virology 39, 562 (1969)
13. Simon, M.N. and Studier, F.W., J.Mol.Biol. 79, 249 (1973)
14. Godson, G.N. and Sinsheimer, R.L., Biochim.Biophys.Acta 149, 476 (1967)
15. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J.Biol.Chem. 193, 265 (1951)
16. Wright, M., Buttin, G., and Hurwitz, J., J.Biol.Chem. 246, 6543 (1971)
17. Goldmark, P.J. and Linn , S., J.Biol.Chem. 247, 1849 (1972)
18. Kerr, C. and Sadowski, P.D., J.Biol.Chem. 247, 305 (1972)
19. Grippo, B. and Richardson, C.C., J.Biol.Chem. 246, 6867 (1971)
20. Studier, F.W., Science 176, 367 (1972)
21. Rahmsdorf, H.J., Pai, S.H., Ponta, H., Herrlich, P., Roskoski Jr., R., Schweiger, M., and Studier, F.W., Proc.Natl.Acad.Sci.USA 71, 586 (1974)
22. Schlegel, R.A. and Thomas Jr., C.A., J.Mol.Biol. 68, 319 (1972)
23. Zissler, J., Signer, E., and Schaefer, F., in "The Bacteriophage Lambda", ed. Hershey, A.D., Cold Spring Harbour Laboratory, New York, pp. 455 (1971)
24. Unger, R.C. and Clark, A.J., J.Mol.Biol. 70, 539 (1972)
25. Sakaki, Y., Karu, A.E., Linn , S., and Echols, H., Proc. Natl.Acad.Sci.USA 70, 2215 (1973)
26. Enquist, L.W. and Skalka, A., J.Mol.Biol. 75, 185 (1973)
27. Tanner, D. and Oishi, M., Biochim.Biophys.Acta 228, 767 (1971)