Adenovirus-Induced Inhibition of Cellular DNase

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During the productive infection of KB cells by adenovirus type 5 (Ad5), there is a progressive decrease in the level of cellular DNase activity towards single-stranded DNA, in contrast to DNA polymerase which remains relatively constant throughout the infection. This decrease is prevented by the inhibition of protein synthesis by cycloheximide. The inhibition of DNase activity does not occur after infection by Ad5 *ts125*, a DNA-negative mutant which fails to induce the adenovirus-specific DNA binding protein. In contrast, infection by Ad5 *ts36*, a DNA-negative mutant which complements *ts125*, does result in decreased levels of DNase. A mechanism is discussed in which the DNA binding protein protects viral replicative intermediates from degradation by cellular DNase.

The replication of adenovirus DNA in human cells involves intermediate structures which contain extensive single-stranded regions (21, 23, 27, 29). These replicative intermediates could be vulnerable to attack by single-strand-specific DNase, and hence, active DNase in the cell might be inhibitory to adenovirus DNA replication. In this paper we report the observation that early after infection by adenovirus type 5 (Ad5), there is a decrease in the level of DNase activity which can be detected in cell extracts. Furthermore, this decrease does not occur after infection by a mutant of Ad5 which is defective in viral DNA replication.

Radioactive adenovirus DNA was used as substrate for DNase assays. Cultures of adenovirus-infected KB cells were labeled with 10 μ Ci of [3 H]thymidine (54 Ci/mmol) per ml at 9 h after infection. The cells were harvested at 48 h after infection and broken by sonic treatment. The virus was purified by fluorocarbon extraction and equilibrium centrifugation in CsCl (24); the DNA was extracted by treatment of the purified virus with sodium dodecyl sulfate and Pronase, followed by phenol extraction. All DNA preparations used had a ratio of optical density at 260 nm to optical density at 280 nm of at least 1.7.

For the assay of DNase activity, the reaction mixture (0.3 ml) contained 2 nmol (DNA nucleotide) of alkali-denatured 3 H-labeled adenovirus DNA (5×10^4 cpm/nmol), 50 mM glycine (pH 9.0), 3.3 mM $MgCl_2$, 10 mM mercaptoethanol, and 0.2 mg of bovine serum albumin per ml. After incubation for 30 min at 37°C, 0.2 ml of salmon sperm DNA (2.5 mg/ml) and 0.5 ml of 10% trichloroacetic acid were added. After centrifugation at $10,000 \times g$ for 10 min, 0.5 ml of the supernatant fluid was removed, and the radio-

activity was determined in a scintillation vial containing 4 ml of ACS scintillation fluid (Amersham/Searle). One unit of enzyme is defined as the amount catalyzing the conversion of 1 nmol of DNA to an acid-soluble form in 30 min at 37°C.

Two types of mammalian DNase which exhibit an alkaline pH optimum and a preference for single-stranded DNA have been described. DNase III is an exonuclease which has been detected in many different mammalian tissues (7, 10, 12, 17), as well as in HeLa cells (3), but has been purified extensively only from rabbit tissue (12). DNase VI is an endonuclease which has recently been purified from human (20) and mouse ascites cells (18). These enzymes can be distinguished by sedimentation analysis of partially hydrolyzed DNA, and by size analysis by paper chromatography (5) of the acid-soluble product. These tests have shown that the DNase assay which we have used, when applied to KB cell extracts, measured both DNase III and DNase VI activities, and that both are present in KB cells.

During the infection of KB cells by Ad5, there is a progressive decrease in DNase activity towards single-stranded DNA (Fig. 1). Sedimentation and product analysis have indicated that both DNase III and DNase VI activities decrease after infection. In contrast to this loss of DNase activity, DNA polymerase activity remains at about the same level throughout the infection (Fig. 1). Removal of more than 80% of the nucleic acids from cell extracts by phase separation does not alter the specific activity of DNase or the relative amounts detectable in infected or mock-infected cells (Table 1).

The decreased DNase activity after infection

could be the result of the generalized shutoff of cellular protein synthesis by adenovirus (8) with resulting decay of the enzyme. To examine this, the effect of inhibition of protein synthesis by cycloheximide was measured, both on the level of DNase and on the effect of adenovirus infection on that level. The results with mock-infected cells (Table 2) show that DNase and DNA polymerase turn over to about the same extent after inhibition of protein synthesis by cycloheximide. In contrast, viral infection results in a sharp decrease in DNase but not in DNA polymerase activity. The loss of DNase due to

virus infection is, therefore, not due to turnover following a generalized shutoff of cell protein synthesis by the virus. The results of Table 2 also show that in the presence of cycloheximide, viral infection does not result in decreased levels of DNase compared with cells mock infected in the presence of cycloheximide. The simplest interpretation of this result is that protein synthesis is required for the decrease in DNase activity after adenovirus infection.

To further clarify the role of the virus in the effect on cellular DNase, temperature-sensitive mutants of Ad5, which are defective in viral DNA replication (2, 33), have been examined for their ability to cause the decrease in cell DNase activity at the nonpermissive temperature. In the experiment shown in Table 3, the specific activity of DNase in extracts of cells infected by Ad5 wild type, *ts36*, or *ts125* at 32°C and 41°C, was compared to that in extracts of mock-infected cells. The results show that *ts125* is defective in the inhibition of DNase at the nonpermissive temperature. In contrast, infection by *ts36* at the nonpermissive temperature does re-

FIG. 1. Decrease in DNase activity after adenovirus infection. At the times indicated after infection of KB cells with Ad5, cells were washed with phosphate-buffered saline, suspended in 0.1 M Tris-hydrochloride buffer (pH 8.1)–2 mM mercaptoethanol (at approximately 5×10^7 cells per ml), and stored at -20°C . After thawing and sonic irradiation in a Bronson Sonifier for 3 min at 4 A in 20-s pulses at 0°C , the extract was centrifuged for 90 min at $10,000 \times g$ in the Sorvall. The supernatant was assayed for DNase and DNA polymerase activity. The DNase assay is described in the text. DNA polymerase was assayed as described previously (4). Protein concentration was determined by the method of Lowry et al. (13). (○) DNase; (■) DNA polymerase.

TABLE 1. Effect of removal of nucleic acids on DNase activity^a

KB cells	DNase activity (U/mg)			Absorbancy at 260 nm	
	Mock infected	Infected	In-fected/mock infected	Mock infected	Infected
Extract	0.16	0.07	0.43	86	112
Phase-separation supernatant	0.14	0.06	0.43	12	15

^a Extracts of KB cells were prepared at 20 h after mock infection or adenovirus infection as described in the legend to Fig. 1. The extracts were subjected to phase separation with dextran T500 and polyethylene glycol as described previously (5). The specific activity of DNase as well as the absorbancy at 260 nm was determined for the extracts and phase-separation supernatants.

TABLE 2. Effect of cycloheximide on the level of DNase^a

KB cells	DNase (U/mg)			DNA polymerase (U/mg)		
	–cyclo ^b	+cyclo	+cyclo/–cyclo	–cyclo	+cyclo	+cyclo/–cyclo
Mock infected	0.60	0.34	0.6	0.08	0.05	0.6
Ad5 infected	0.16	0.31	1.9	0.11	0.08	0.7
Infected/mock infected	0.27	0.91		1.4	1.6	

^a KB cells were mock infected or infected with wild-type Ad5. To half the cultures, cycloheximide was added at 2 h after infection or mock infection at a final concentration of 30 $\mu\text{g}/\text{ml}$. At 25 h after infection or mock infection, extracts were prepared, and the specific activities of DNase and DNA polymerase activity were determined (see text and Fig. 1).

^b cyclo, Cycloheximide.

TABLE 3. Inhibition of DNase after infection by adenovirus DNA-negative, temperature-sensitive mutants^a

Virus	DNase specific activity (% of mock infected)		
	Infection at:		
	32°C	41°C	32°C/41°C
Wild type	39	22	1.8
ts36	51	44	1.2
ts125	40	106	0.4

^a KB cells were infected or mock infected and incubated at 32 or 41°C. At 48 h (32°C cultures) or 20 h (41°C cultures) after infection, extracts were prepared, and DNase was assayed as described in the text.

sult in decreased DNase activity, although the decrease does appear to be somewhat smaller. Both mutants behave like wild type after infection at 32°C. The infectivity and temperature sensitivity of DNA synthesis of the mutant viral stocks used for these experiments were confirmed by measuring the synthesis of viral (31S) DNA in vivo in infected cells at permissive and nonpermissive temperatures.

These results indicate that the decrease in DNase appears to require active ts125 gene product, which has been shown to be the adenovirus-specific DNA binding protein (31), which binds specifically to single-stranded DNA (30). The simplest interpretation of the data is that this adenovirus-coded DNA binding protein acts as an inhibitor of DNase by binding to the single-stranded DNA substrate. In this regard, inhibitory activity towards several DNases has been demonstrated for the *Escherichia coli* DNA unwinding protein (14, 16). This activity could at least in part explain the requirement for the DNA binding protein for adenovirus DNA synthesis in vivo and the DNA-negative phenotype of ts125. Since adenovirus replicative intermediates contain extensive regions of single-stranded DNA which might be susceptible to degradation by DNase, failure to inhibit DNase after infection by ts125 might result in rapid destruction of viral replicative intermediates. Attempts to directly detect a DNase inhibitor in infected cells have been complicated by the presence of an inhibitor in uninfected cells (Frenkel and Nass, in preparation). This inhibitor is also a DNA binding protein and contaminates preparations of the adenovirus-specific DNA binding protein. We are attempting to separate the two proteins to assess the ability of the viral DNA-binding protein to directly inhibit DNase.

Viral modulation of cellular enzyme activities appears to be an important feature of the interaction between bacteriophages and their host bacteria. A variety of phages have been shown to induce inhibitors of a variety of cellular en-

zymes (6, 9, 15, 22). Cellular DNases in particular appear to be a target of phage-induced inhibition (1, 11, 19, 25, 26, 28, 32). Evidence from these systems suggests that each case of inhibition represents the individual phage's response to its individual requirements for replication. This type of viral regulatory mechanism is distinct from the overall effect of the virus on the macromolecular synthesis of the cell, allowing the virus to specifically alter the cellular environment to make it more compatible with viral replication. The results presented in this paper represent the first observation of what may be a similar phenomenon in the interaction of animal viruses with their host cells. Further work will be required to determine how general this phenomenon is for animal viruses both in terms of the viruses which cause such effects and the cellular enzymes affected.

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