NUCLEASES OF VIRUS-INFECTED ANIMAL CELLS*

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Infection of Hela cells with cowpox but, apparently, not Rabbitpox causes a marked increase in a DNAase activity that degrades native DNA at alkaline pH values (McAuslan, 1965). The purpose of this communication is to describe the viral induction of yet another nuclease —— a DNAase that degrades thermally denatured DNA. In addition we demonstrated that the magnitudes of induced DNAase activities depend markedly on both the cell type and the virus strain used. Preliminary results of studies with other DNA viruses are presented.

Chick embryo fibroblasts (CEF) were grown as monolayers in Hank's medium containing 0.25% Lactalbumin hydrolysate and 5% calf serum (preheated 56°C, 30 mins). The assay for DNAase activity was essentially as described previously (McAuslan, 1965); for DNAase degrading thermally denatured DNA the assay was modified as described under fig. 1. To prepare the substrate for the latter enzyme, E. coli DNA-P^{32} (70 µg/ml in 0.01M Tris pH 7.2) was heated at 100°C for 10 minutes then rapidly chilled to 0°.

The purified viruses used were the Utrecht strain of Rabbitpox (RP) and the Brighton strain of Cowpox (CP), both of which were grown in the chick chorio-allantoic membrane, Adenovirus type II grown in KB cells, the WR strain of vaccinia virus grown in Hela cells and the McIntyre strain of Herpesvirus grown in Monkey kidney cells. All other relevant techniques and materials have been described or referred to in a prior communication (McAuslan, 1965).

Six hours after infection of spinner cultured Hela-S^3 cells with CP at an input multiplicity of 10 PFU per cell, there is a 5-10 fold increase in the activity of a DNAase that degrades thermally denatured DNA. This will be referred to as "acid"

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DNAase since it exhibits high activity at pH5. The kinetics of the increase in "acid" DNAase activity are compared with those of "alkaline" DNAase (fig. 1).

The induced "acid" DNAase activity decreased with increasing the pH above 5.0 and is minimal at about pH 7.5. Crude cell extracts retain full activity even after being held at 60°C for 30 minutes at pH7.0. Mg^{++} ions above a concentration of 0.3mM markedly inhibit both pre- and post-infection enzyme activities.

The increase in "acid" DNAase appears to represent de novo enzyme synthesis rather than activation of a preexisting enzyme since puromycin hydrochloride (25 ug/ml) inhibits the post-infection increase in activity (fig. II). In contrast to the puromycin inhibition of the synthesis of poxvirus-induced thymidine kinase (McAuslan, 1963), the puromycin inhibition of the increase in "acid" DNAase activity

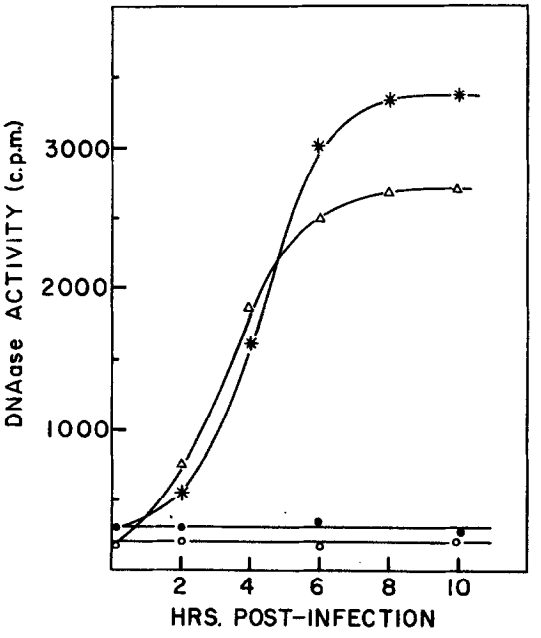

Fig. I. Increase in "acid" DNAase and "alkaline" DNAase in CP-infected Hela cells. (O) Normal cell extracts, "alkaline" DNAase; (●) Normal cell extracts, "acid" DNAase; (Δ) infected cell extracts, "alkaline" DNAase; (✱) infected cell extracts, "acid" DNAase. Assay conditions for "acid" DNAase: the reaction mixture (350 µl) contained heat denatured DNA-P^{32} (25 µg, 20,000 CPM); $MgCl_2$ (0.1 µmoles); cell extract (10 µl; 100 mg protein). The reaction mixture was adjusted to 0.1 M sodium acetate pH5 and incubated for 40 minutes at 37°. The reaction was terminated by chilling, carrier DNA (0.1 ml containing 0.25 mg DNA and 0.5 mg bovine serum albumin) and 0.5N $HClO_4$ (0.45 µl) was added. After 3 minutes at 0°, the mixture was centrifuged (3 mins, 10,000 g) and the supernatant transferred to a planchette for determination of radioactivity. Activity is expressed as CPM rendered acid soluble under these assay conditions.

is not reversible. This suggests that the messenger RNA for "acid" DNAase is comparatively unstable.

Rapid inhibition of the increase in "acid" DNAase activity following addition of actinomycin D (5 ug/ml) supports this suggestion (fig. III). Similar results have been obtained for "alkaline" DNAase.

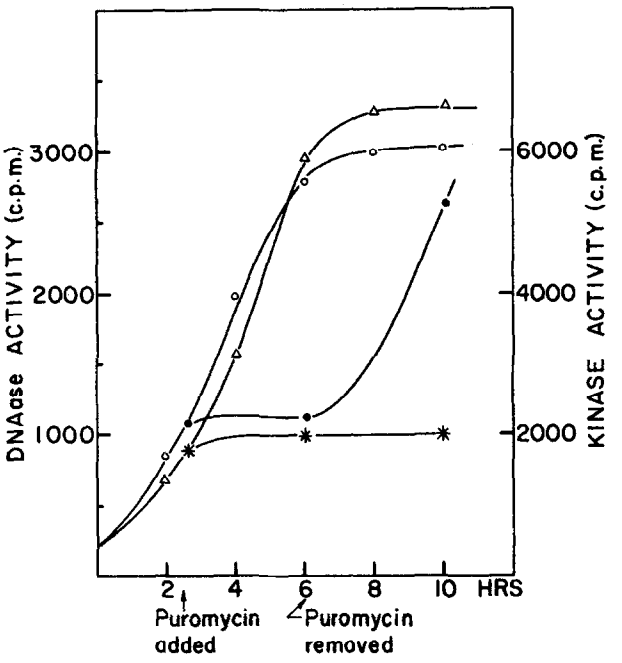

Fig. II. Irreversible Puromycin inhibition of the post-infection increase in "acid" DNAase activity. (Δ) "Acid" DNAase increase post-infection; (✳) "acid" DNAase increase after puromycin HCl (25 µg/ml) was added at 2 1/2 hours then washed out at 6 hours post-infection; (○) thymidine kinase activity post-infection; (●) thymidine kinase activity on same extracts as for (✳).

Several combinations of virus strain and cell type have been compared for the magnitude of the induced "acid" and "alkaline" DNAase activities. Cells were infected with virus at the same input multiplicity in each case and cell extracts were compared for activity either 6 hours (CP and WR) or 10 hours (RP) post-infection (Table I).

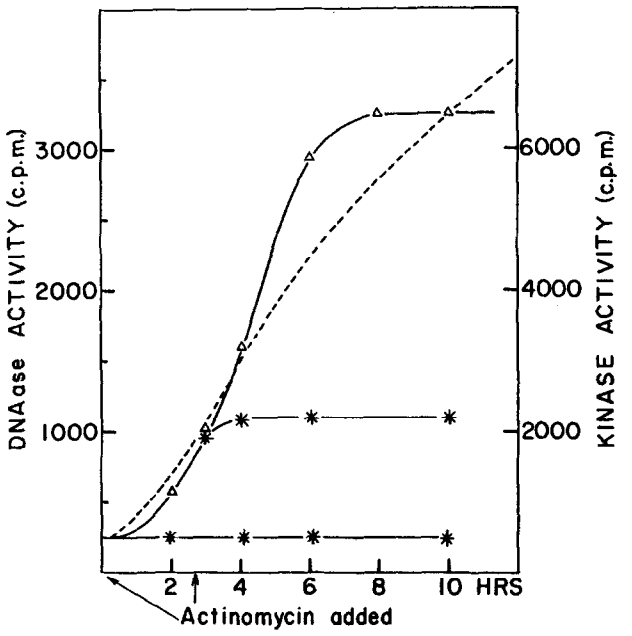

Fig. III. Effect of Actinomycin D on CP induction of "acid" DNAase. (△) Increase in "acid" DNAase post-infection; (✱) increase in "acid" DNAase after addition of Actinomycin D (5 μg/ml) as indicated. The amount of Actinomycin D retained in the cell extract did not significantly influence the assay of DNAase activity. (- - -) Thymidine kinase activity of extracts of infected cells treated with Actinomycin D at 2 1/2 hours post-infection.

Table I

CELL	CP		RP		WR	
	Native DNA pH 9.2	Denatured DNA pH 5	Native DNA pH 9.2	Denatured DNA pH 5	Native DNA pH 9.2	Denatured DNA pH 5
Hela	20 x	10 x	Neg	Neg	5 x	Neg
CEF	7 x	Neg	2 x	Neg	2 x	Neg

Table I. Increase in DNAase activities of different cells after infection with different poxviruses. Increase in specific activity is represented as a multiple of the corresponding specific activity of uninfected cell extracts. Increases of 50% or less were scored as negligible (neg).

These studies have been extended to adenovirus type II- infected KB cells and Herpesvirus-infected Hela -S cells. No increase in any DNAase (single or double-stranded substrate at acid, neutral or alkaline pH values could be demonstrated after adenovirus infection. On the other hand infection of Hela-S3 monolayers

with Herpes virus (input multiplicity of about 1 PFU. per cell) caused a striking increase in at least two DNAase activities. Activities increased with time were maximal about 8 hours post-infection (Table II) and remained constant for at least a further 4 hours.

Table II

pH of Assay	7.2	7.8	8.5	9.2	9.5	10.0
Normal Extract + Native DNA	264	200	264	230	---	180
Normal Extract + Denatured DNA	264	100	784	1000	---	6160
Herpes-infected Extract + Native DNA	2314	1770	4672	9510	---	6160
Herpes-infected Extract + Denatured DNA	1800	4000	4572	9474	9510	9352

Assay conditions as described by McAuslan (1965).

Discussion. An increase in the activities of two DNAases is clearly demonstrated for Hela cells infected with cowpox virus. These are an "alkaline" DNAase that degrades double-stranded DNA (McAuslan, 1965) and an "acid" DNAase that degrades single-stranded DNA. Preliminary studies with infected cells fractionated by a modification of the Dounce technique indicate that the induced "alkaline" DNAase appears to be located predominantly in the nucleus and the induced "acid" DNAase is located exclusively in the cytoplasm. In marked contrast to the messenger stability of poxvirus induced thymidine kinase (McAuslan, 1963) the messengers for DNAases are unstable. The results in Fig. III indicate that the effective half life of the mRNA for "acid" DNAase is of the order of 1 hour. Despite the fact that synthesis of DNAase and thymidine kinase is terminated simultaneously in poxvirus-infected Hela cells, one might expect from the difference in messenger stability that the control of synthesis would be different for the two enzymes. In fact, it can be shown for at least one poxvirus-cell system that the synthesis of "alkaline" DNAase is terminated even before the synthesis of thymidine kinase can be detected (McAuslan, unpublished data). The fact that the increase in DNAase activity requires protein synthesis indicates that the increase is not due merely to activation of lysosomal enzymes as suggested by Allison and Sandelin (1963). At least two "alkaline" DNAases are induced by Herpes virus in Hela cells.

We have not yet established if these represent two distinct enzymes or the one enzyme with two different activities. No increase in "acid" DNAases after Herpes infection could be detected.

Further experiments are required to determine if the lack of increase in "acid" DNAase after infection with some virus strains is due to failure to synthesize enzyme or to lability of the induced DNAase. Until this point is clarified and the mechanism of both DNAases is established, speculation on their possible role in virus replication would be premature.

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