THE NATURE OF POXVIRUS-INDUCED DEOXYRIBONUCLEASES*

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Three nuclease activities can be readily demonstrated in crude extracts of cultured HeLa cells: one acting optimally at pH 9.2 on denatured DNA, one at pH 5 on native DNA and one at about pH 8.5 on native DNA. Infection of HeLa cells with poxvirus causes the induction of at least two new deoxyribonuclease activities. One of these ("alkaline" DNAase) is most active at pH 9.2 and is specific for native DNA (McAuslan, 1965). The other induced enzyme ("acid" DNAase) is most active at pH 5 and is specific for heat-denatured DNA (McAuslan et al., 1965). Knowledge of the mode of action of these enzymes is a prerequisite for speculation on their possible role in the replication of viral DNA. We have therefore partially characterized the induced DNAases according to whether their action is predominantly exo- or endonucleolytic. Studies on the pre-infection nucleases of the HeLa cell are presented for comparison.

Materials and Methods: The procedure for measuring endonuclease acting on denatured DNA was a slight modification of the nitrocellulose membrane filter technique described by Geiduschek and Daniels (1965). For this assay and for the assay of DNAase activity by measurement of the formation of acid-soluble products, DNA labelled with tritiated thymidine was prepared by Marmur's method (1961) from E. coli T^- grown to a limit in tris-glucose medium containing 1 mc. Thymine-H^3 per liter. Endonuclease acting on native DNA was measured by viscometry (Eron and

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McAuslan, 1966). Calf thymus DNA (Sigma, Highly Polymerized) was used in this case.

The compositions of the reaction mixtures and the amounts of enzyme added were essentially the same as those described previously (McAuslan, 1965, McAuslan et al., 1965) with the following exceptions: The concentration of DNA in the reaction mixture assayed by the membrane filter technique was reduced to one fifth, and the concentration of DNA in the reaction mixture assayed by viscometry was doubled. Also, all the reactants for the latter assay were scaled up approximately twenty-fold for efficient operation of the viscometer.

Extracts for the enzyme assay were prepared ten hours after infection of cells with cowpox virus. All other relevant techniques, including conditions for heat denaturation of the DNA, have been described in the previously mentioned communications.

Results: Alkaline DNAases (Native DNA, pH 9.2). In uninfected HeLa extracts this activity, even at its optimum pH of 8.5, is low as measured by the production of acid-soluble material. There is, however, significant endonuclease activity as measured by viscometry (see fig. 1a). Extracts

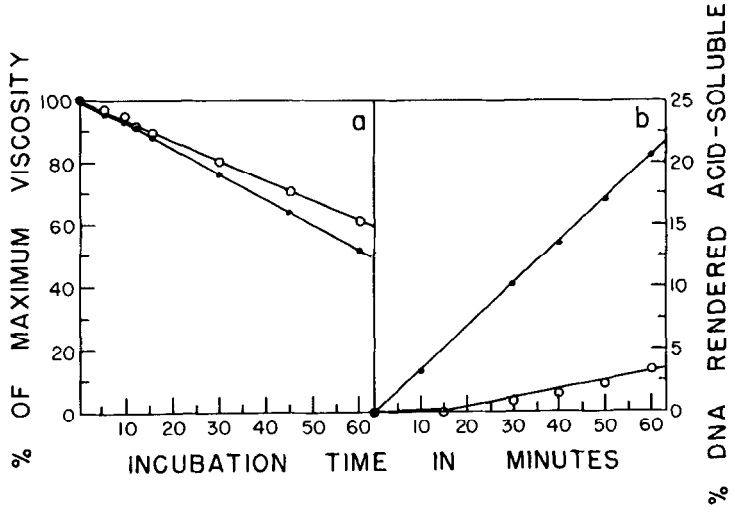

Fig. 1. DNAase activity assayed with native DNA at pH 9.2. (○) normal cell extracts; (●) infected-cell extracts. a) assayed by viscometry; b) assayed by measurement of acid-solubles.

of cells infected for 10 hours with cowpox or rabbitpox show a marked increase in the capacity to degrade native DNA to acid-soluble products with no corresponding increase in endonuclease activity (fig. 1a, 1b). Aliquots of the reaction mixture, in which E. coli DNA-H^3 was used, were chromatographed on Whatman No. 1 paper with the solvent described by Moscarello et al. (1961). The amount of labelled TMP or TdR liberated by the extracts was used to compare their exonuclease activity (Weissbach and Korn, 1963). From Table I, it is obvious that the increased DNAase activity of virus-infected cells coincided with an increased rate of liberation of mononucleotides.

Table I. Liberation of thymidylate and thymidine from native DNA at pH 9.2 by normal and infected cell extracts.

	% of total DNA	
	Normal	Infected
Acid Soluble Products	14	90
Zone in chromatogram		
Thymidylate	0.1	14
Thymidine	0.6	10
% of Acid Soluble Products that are dTMP or TdR	5	30

Alkaline DNAase (Denatured DNA, pH 9.2). There is no significant

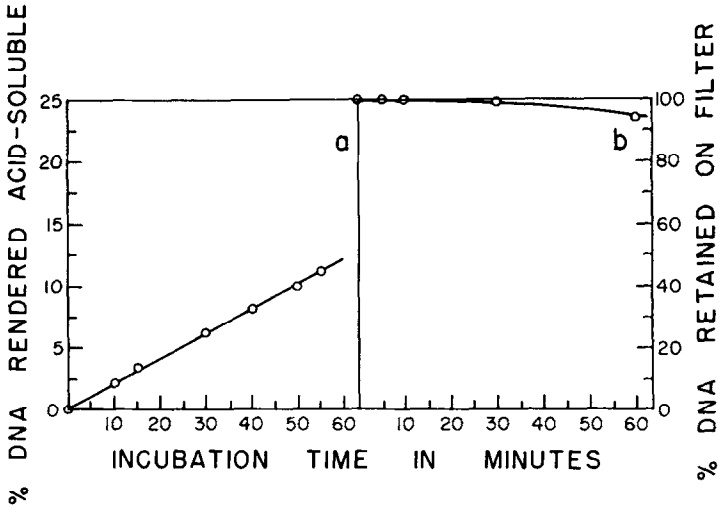

Fig. 2. DNAase activity assayed with heat-denatured DNA at pH 9.2. (O) normal or infected cell extracts. a) assayed by measurement of acid-solubles; b) assayed by retention of DNA on a membrane filter.

increase in this type of activity after poxvirus infection. Endonuclease activity measured by the membrane filter technique was negligible, but the rate of liberation of acid-soluble components from DNA was high (fig. 2). McAuslan and Kates have observed a 1 1/2-2 fold increase in a DNAase (denatured DNA, pH 7.8) after infection of cell with cowpox or rabbitpox by using the assay described by Jungwirth and Joklik (1965). This activity has not yet been further characterized.

Acid DNAase (Native DNA, pH 5). There is a comparatively high DNAase activity of this type from uninfected cell extracts as assayed either by viscometry or by the formation of acid-soluble products (fig. 3). This activity is probably the same type as that found in many vertebrate tissues (discussed by Bernardi and Cordonnier, 1965). There is no increase in the activity following poxvirus infection, as measured by either technique.

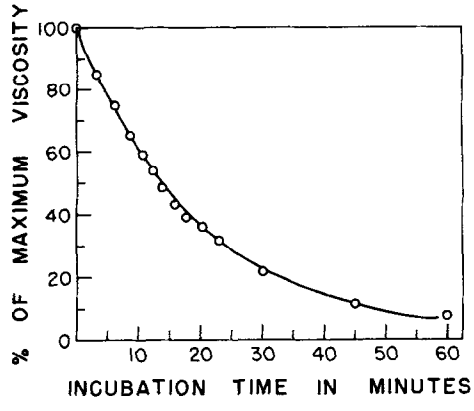

Fig. 3. DNAase activity assayed with native DNA at pH 5. (O) normal or infected cell extracts assayed by viscometry.

Acid DNAases (Denatured DNA, pH 5). Under the given conditions of substrate and pH, uninfected cell extracts exhibit endonuclease activity. The capacity of infected cell extracts to degrade the substrate to acid-soluble components is greatly enhanced (fig. 4a) (McAuslan et al., 1965), but the endonuclease activity is not increased (fig. 4b).

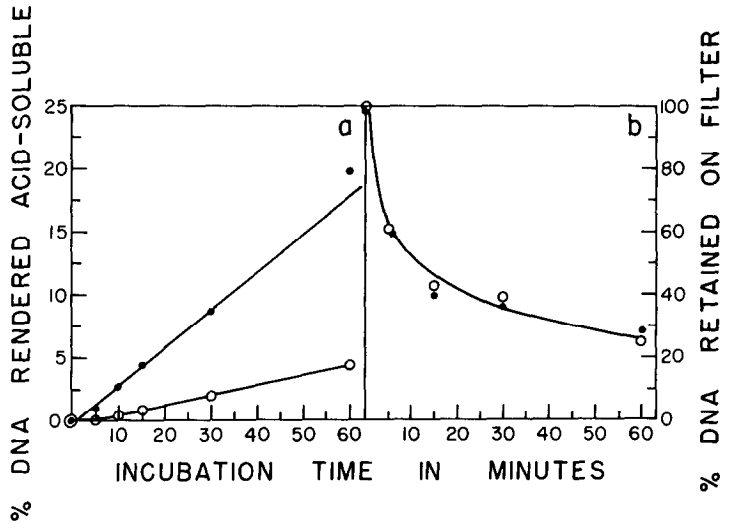

Fig. 4. DNAase activity assayed with heat-denatured DNA at pH 5. (O) normal cell extracts; (●) infected cell extracts. a) assayed by measurement of acid-solubles; b) assayed by retention of DNA on membrane filter.

Discussion: Despite the use of crude cell extracts, one can determine if the post-infection increase in the capacity of cell extracts to degrade DNA to acid-soluble components is accompanied by an increase in either exo- or endo-nuclease activity. If both endo- and exo-nucleolytic activities had increased concurrently, it could be argued that only endo-nucleolytic activity had increased and the apparent enhanced capacity to degrade DNA to acid-soluble components was a secondary effect due to pre-existing exonuclease having more DNA ends to act upon. However, in no case was there an increase in the capacity of extracts to decrease the viscosity of DNA solution attributable to endonuclease activity, along with the post-infection increase in the capacity to degrade DNA to acid-soluble components. Both virus-induced enzymes are exonucleases. This conclusion is strengthened in the case of the induced "alkaline" DNAase (degrading native DNA) by demonstrating a post-infection increase in the capacity of the cells to liberate mononucleotides from DNA.

A summary of our knowledge of the major pre- and post-infection DNAases in the HeLa cell is shown in Table II.

Table II

Source	DNA Preferred Physical State	pH for Max. Activity	Predominant Action
Uninfected HeLa	Native	8.5	endo-
Pox-infected HeLa	Native	9.2	exo-
Uninfected HeLa	Denatured	9.2	exo-
Pox-infected HeLa	Denatured	7.8	not examined
Pox-infected HeLa	Denatured	5	exo-
Uninfected HeLa	Denatured	5	endo-
Uninfected HeLa	Native	5	endo-

Since previous publications on DNAases we have been able to detect all three induced DNAases after infection of HeLa cells with cowpox, rabbitpox, or the CL-R and WR strains. Failure to detect increases in DNAases after rabbitpox infection as previously noted (McAuslan, 1965, McAuslan et al., 1965) is probably due to instability of the enzymes under certain conditions. This point will be elaborated in a future publication.

The experiments described above and in previous communications from the laboratory do not exclude the possibility that other nuclease are present in either the normal or poxvirus-infected cells, and our conclusions concerning the mode of action of the nucleases require support from experiments on the purified enzymes. Yet to be answered is whether increased nuclease synthesis represents an expression of the host's synthetic capacities or the direct expression of the virus genome. In this respect, it will be of interest to compare the corresponding nucleases (native DNA, pH 9.2) induced by Herpes and poxvirus (McAuslan et al., 1965).

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