DEOXYRIBONUCLEASE ACTIVITY OF NORMAL AND POXVIRUS-INFECTED HELA CELLS*

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An increase in the deoxyribonuclease activity of L-cells infected with Vaccinia virus was noted by Hanafusa (1960). More recently Magee (1963, 1964) has been unable to detect such an increase in activity. Clarification of these controversial findings may be relevant to the study of viral replication and, in particular, to the study of poxvirus-induced DNA polymerase (Magee, 1962). We therefore undertook a re-examination of the effect of poxvirus infection on DNAase activity.

This communication shows that a striking increase in DNAase activity of Cowpox-infected Hela-S_3 cells is demonstrable. In addition, we find that the induced enzyme activity has properties that distinguish it from the corresponding activity of uninfected Hela-S_3 cells. However, the ability to induce DNAase activity does not appear to be a property of all poxviruses.

The maintenance of cells, procedures for their infection with virus, the preparation of cell extracts and the method for determination of protein concentration have been described (McAuslan and Joklik, 1962). The virus used was the Brighton strain of cowpox (CP) or the Utrecht strain of rabbitpox (RP) purified by methods described by Joklik (1962). The substrate for the enzyme assay was DNA-P^{32} prepared by Marmur's method (1961) from E-coli B grown to a limit in tris-glucose medium containing 1 mc $P^{32}O_4$ per litre. Acid soluble counts in the product were practically

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eliminated by treatment with Norit (Lehman, 1960). For some experiments DNA-P^{32} (70 μg) in 1.0 ml 0.02 M NaCl containing 0.025M tris pH7.5 was heated (10 mins. 100°C) then chilled to 0°. For comparison of the properties of DNAase of normal and infected cells, extracts were prepared 7 hours post-infection.

The kinetics of the increase in DNAase activity of infected-cell extracts under the assay conditions are shown in Fig. 1. Similar results have been obtained with cells infected with the Connaught Laboratories strain of vaccinia virus. No significant change in the DNAase activity of RP infected cells could be detected although thymidine kinase was induced.

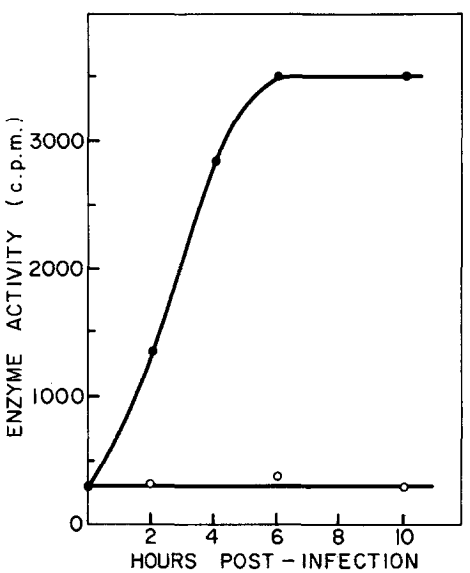

Fig. 1. Increase in DNAase activity of CP-infected Hela cells. (O) Normal cell extracts; (●) infected-cell extracts. Assay conditions: The reaction mixture (350 ul) contained native DNA-P32 (25 μg; 20,000 CPM); MgCl2 (2 μ moles); glycine-NaOH pH 8.5 (33 μmoles); cell extract 25 μl, 250 μg protein). The mixture was incubated for 40 minutes at 37° then chilled. Carrier DNA (0.1 ml containing 0.25 mg DNA and 0.5 mg bovine serum albumin) and 0.5 N $HClO_4$ (0.45 ml) was added. After 5 minutes at 0°, the mixture was centrifuged (3 mins. 10,000 xg) and the supernatant transferred to planchettes for determination of radioactivity. The assay was linear within increasing protein over the range 50 - 500 μg and with time, for at least 60 minutes. Activity is expressed as cpm rendered acid soluble under these assay conditions.

The influence of the physical state of the substrate on the reaction rate demonstrates a difference in substrate requirements of the activities of normal and CP-infected cell extracts (Fig. 2).

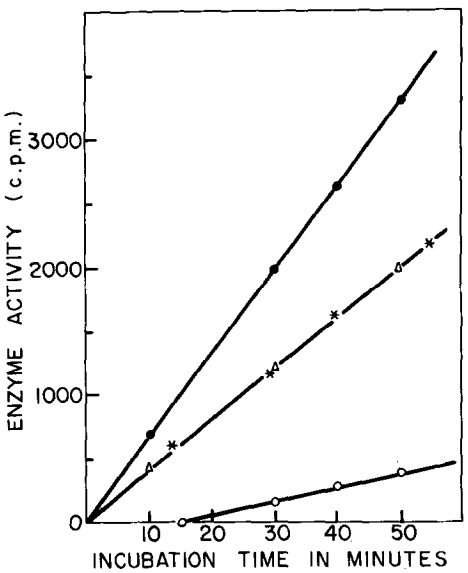

Fig. 2. Effect of the physical state of the substrate on DNAase activity of normal and CP-infected cell extracts. (●) infected cell extracts plus native DNA; (✱) infected-cell extracts plus thermally denatured DNA; (Δ) normal cell extracts plus thermally denatured DNA; (○) normal cell extracts plus native DNA.

To see how many distinguishable enzyme activities are actually being measured, thermal stability curves were plotted for the four cases outlined in Fig. 2. The results (Fig. 3) indicate that probably only the one enzyme activity is being measured by using thermally denatured DNA. The unusual thermal stability curve at 47° for infected cell DNAase assayed with native DNA, is not due to the presence of excess enzyme in the assay.

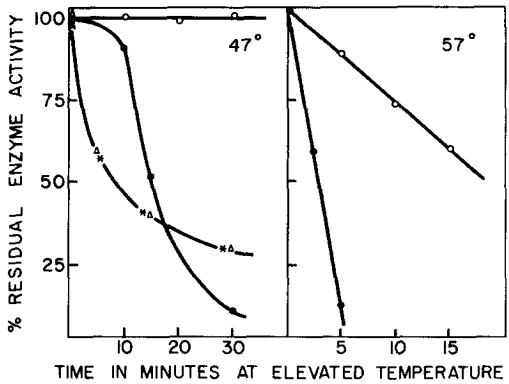

Fig. 3. Thermal stability of DNAase activity of Normal and Infected-cell extracts. Extracts at the same protein concentration (pH 7.5) were held for various periods at either 47° or 57° then chilled before assaying as described under Fig. 1. (O) normal extracts plus native DNA; (Δ) Normal extracts plus denatured DNA; (●) infected extracts plus native DNA; (✱) infected extracts plus denatured DNA.

The influence of pH on enzyme activity is shown in Table I.

TABLE I

DNA STATE	ENZYME EXTRACT	5	7.0	7.8	8.5	9.2	9.6	10.0	10.6
Native	Normal	1050	375	300	350	290	200	180	160
Native	Infected	1100	750	2000	3325	4695	2990	360	145
Denatured	Normal	350	310	50	870	2250	1600	625	50

Assays were essentially as described for Fig. 1. Buffers used were 0.1M Acetate at pH5: 0.1M tris HCl in the range 7 to 9.2 or 0.1M glycine NaOH in the range 8.5 to 10.6.

The "alkaline" DNAase of normal and infected-cell extracts were compared for their sensitivity to various inhibitors (Table II).

Extracts of uninfected Hela cells assayed with native DNA, show low "alkaline" DNAase activity. The corresponding "acid" DNAase activity (pH5) is approximately three times that of the alkaline DNAase. Omission of Mg^{++} ions markedly increases this "acid" DNAase activity. No post infection change in "acid" DNAase was detected. At pH 9.2, with

TABLE II

	% Maximal Activity	
	Normal	Infected
Standard assay (pH 8.5, native DNA)	100	100
Standard assay + 1μg Actinomycin D	100	50
Standard assay + 10 μg Actinomycin D	75	10
Standard assay minus Mg^{++}, plus Mn^{++}	150	40
Standard assay plus 500 μg E. coli s-RNA	40	100

native DNA as substrate, up to 20 fold increases in the DNAase activity of CP-infected cells are demonstrable. The kinetics of this increase in activity closely follows those for poxvirus-induced thymidine kinase synthesis, (McAuslan, 1963 a,b). Pre-treatment of cell-extracts with RNAase did not affect the results, indicating that the increase in DNAase activity is not due to viral-induced destruction of an RNA inhibitor of DNAase. In vitro, extracts of normal cells or of RP-infected cells do not inhibit the DNAase activity of CP-infected cell extracts. The increase in alkaline DNAase is at least 400 times greater than could be accounted for as complete adsorption of DNAase activity from the virus inoculum. Further, the "alkaline" DNAase of fertile hen egg membranes (on which virus stocks were grown) is similar in its properties to that activity of uninfected Hela cells.

The results in Fig. 2 show that the induced DNAase has a preferential, perhaps absolute, requirement for native DNA whereas the DNAase activity of uninfected cells is 5 fold higher for heat denatured DNA than for native DNA. The pre- and post- infection DNAase activities can also be differentiated by their response to Mn^{++} ions and to actinomycin D.

A likely explanation for the appearance of the different DNAase activity induced by infection, is that cowpox codes for the synthesis of this

enzyme. The DNAase activity of uninfected- or infected-cell extracts under conditions used to assay DNA polymerase (see Green and Pina, 1962; Magee, 1962) is unlikely to significantly influence the latter reaction. However, the use of a non-DNAase inducing strain (RP) might be preferable for further study of viral induced polymerase. The failure of RP to induce DNAase does not appear to be due to the presence of an inhibitor present in the inoculum or induced by RP infection. We are investigating the possibility that the CP induced DNAase influences the yield of infectious cowpox which, for the Hela cell system, is low compared to the yield of infectious RP.

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