# Deoxyribonucleases in Herpes simplex Virus Type 1 and 2 Infected Primary Rabbit Kidney Cells

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In primary rabbit kidney cells infected with herpes simplex virus four different neutral deoxyribonuclease activities can be detected by means of the deoxyribonuclease assay in DNA-containing polyacrylamide gels following their separation by disc-electrophoresis. The method is suitable to follow independently the change in each activity of the different enzymes using only about $5 \times 10^5$ cells for each assay during the time-course of infection. Under these conditions one enzyme activity is constant, two disappear while the activity of a fourth one present only in infected cells, increases.

DNA virus associated nucleases degrading DNA have been described in many cases [1–12]. Also cells infected with these viruses [12–16] show new or higher deoxyribonuclease (DNase) activities.

Keir and Gold [17], Russell *et al.* [18] and Morrison and Keir [16] detected a neutral deoxyribonuclease in mammalian tissue culture cells which increases after infection of the cells with herpes simplex virus. This enzyme appeared to differ from the host enzyme. McAuslan *et al.* [19] observed an "alkaline" DNase activity in herpes infected HeLa cells. Newton [20] reported the increase of an "acid" DNase 8 h after infection of HeLa cells. Recently Francke *et al.* [21] and Clough [22] described alkaline DNase-activities in baby hamster kidney cells infected with herpes simplex virus type 2 and in Eppstein barr virus producing lymphoblastoid cells. In both cases a potential role of these enzymes during viral replication is discussed. To elucidate the role of DNases for the virus-cell interaction it seems necessary to obtain a valuable test separating the virus enzyme or the virus induced enzyme from the cellular DNases and determining their activity during the course of infection.

In this report we show that it is possible to identify a DNase in Herpes simplex virus infected primary rabbit kidney cells by means of the in situ detection of DNases in DNA containing polyacrylamide gels following their separation by micro disc electrophoresis.

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## Materials and Methods

Primary rabbit kidney cells in 10 cm petri dishes have been used throughout the experiments. The technique of growing cells has been described elsewhere [23].

Strains Lennette (type 1) and D-316 (type 2) have been used [24]. The multiplicity of infection was about 5. Two h after infection the not absorbed virus was washed off and medium containing 10% calf serum was added.

At different times after infection petri dishes containing infected or control cells were removed, scraped into Hanks solution and spun down. After washing two times, the cells were sedimented again and stored at −70 °C until use.

The in situ detection of DNases in DNA containing polyacrylamide gels following electrophoretic separation was performed as previously described [12, 25, 26]. For DNase assay approximately $5 \times 10^5$ cells were suspended in 50 µl spacer gel buffer containing 30% sucrose, 0.5% NP 40 and 10 mM mercaptoethanol. The cells were disrupted by a combination of freezing and thawing three times and incubation at 37 °C for 15 min. 8 µl of this sample were applied to each gel for electrophoretic separation and DNase assay. We used a 5% acrylamide spacer gel (pH 6.7; 0.176 M Tris $H_3PO_4$) and a 13.4% acrylamide separation gel (pH 8.8; 0.177 M Tris $H_2SO_4$) with 0.3 mg/ml DNA. Herring sperm DNA, prepared according to Zahn *et al.* [27] was denatured by heating for 10 min to 100 °C in a boiling water bath and chilling in ice. All operations from gel formation to incubation were carried out at

0 °C. Electrophoresis was performed at 100 μA/gel for 80 min with the anode at the bottom. After the electrophoretic run the gels were incubated in different incubation mixtures for 4 h at 37 °C, stained with gallocyanine-chromalaune and, after destaining with water, the optical density was recorded with a densitometer.

## Results and Discussion

Using the DNase assay in DNA containing polyacrylamide gels, different DNase activities are detectable under acid (0.1 M Na-acetate pH 5.0, 1.5 mM EDTA), neutral (0.1 M Tris-HCl pH 7.4, 10 mM $MgCl_2$, 1 mM $CaCl_2$) and alkaline conditions (0.025 M Tris-HCl pH 8.5, 10 mM $MgCl_2$) in primary rabbit kidney cells. Using acid or alkaline incubation conditions for DNase detection no changes in the DNase pattern could be shown during the time course of infection of primary rabbit kidney cells with herpes simplex virus. Only in the neutral DNase pattern a striking change can be seen during the time-course of infection (Fig. 1). Two neutral DNase activities (Fig. 1 a, d) migrating just towards the anode disappear while an additional DNase activity (Fig. 1 e, f) increases. The enzyme activity which only enters the gel seems to be constant during 24 h. The activity increasing during infections shows a maximum after 8 h and increases again after 14 h after infection (Fig. 2). A similar DNase activity was demonstrable in primary rabbit kidney cells infected with herpes simplex virus type II. These activities do not correspond to the DNase activity induced in baby hamster kidney 21-C 13 cells after infection with herpes simplex virus described by Morrison and Keir [16].

It is not heat sensitive and does not require $Na^+$ for optimal activity. Also the exonucleases described by Francke *et al.* [21] in herpes simplex virus infected cells and the nuclease in Eppstein-Barr virus producing lymphoblastoid cells, isolated by Clough [22] obviously do not correspond to the DNase-activity increasing during the time course of infection. Both activities show an alkaline pH optimum around pH 8.5. Under these conditions the DNase

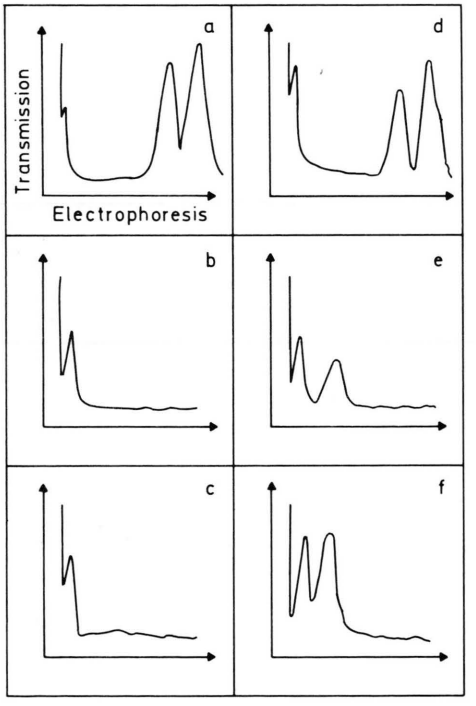


Fig. 1. Detection of DNase in prk cells and in herpes simplex virus infected cells at different times after infection by in situ assay in DNA containing polyacrylamide gels. On each gel containing denatured DNA 8 μl corresponding to $8 \times 10^4$ cells were applied. The electrophoresis was performed at 700 μA/gel for 80 min. After the electrophoretic run, the gels were incubated for 2 h in 0.1 M Tris-HCl pH 7.4, 10 mM $MgCl_2$, 1 mM $CaCl_2$ at 37 °C. After staining with gallocyanine chromalaune and destaining with water, the optical density of the gels were recorded with a densitometer. a, b, c uninfected cells; d, e, f virus infected cells, after 2 h (a, d), 8 h (b, e) and 24 h (c, f).

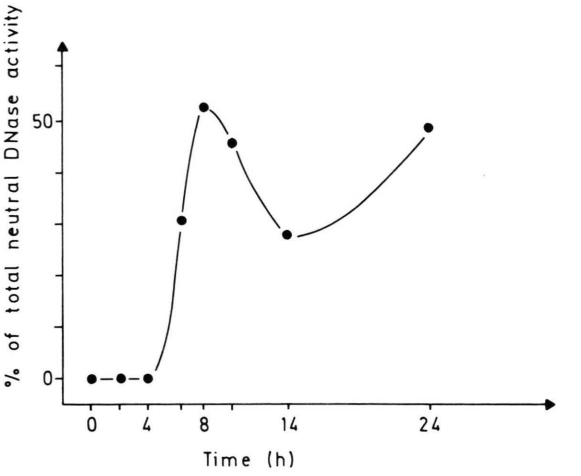


Fig. 2. Time course of herpes simplex virus induced DNase activity in prk cells as determined by in situ DNase assay. Experimental procedures as in Fig. 1. The activity is expressed in percent of the total neutral DNase activity detectable by the microdisc electrophoretic assay.

described here is not detectable. Also the endonuclease from herpes simplex virus, recently characterized by Hoffmann and Cheng [28], differs from the enzyme – activity described above in respect to the inhibitory effect of $Ca^{2+}$ in the presence of $Mg^{2+}$.

The dissapearance of the two neutral DNase activities (Fig. 1 a, d) is not characteristic for virus infected cells. They are not detectable in infected cells at different times after infection (Fig. 1 e, f) but they are also missing in control cells (Fig. 1 b, c). With the results available it can only be supposed, that the decreasing activity may be correlated to a decrease in cellular processes, as for example DNA Synthesis, in the state of contact inhibition. It occurs when the cells have been grown in the petri dishes to be used for infection with viruses. This must be investigated in a separate study.

The results show that there are different DNase activities in herpes simplex virus infected cells, active under the same incubation conditions with partly opposite changes in activity during the time course of the infection. The method described above seems to be suitable to follow independently the change in each activity of the different enzymes using only about $5 \times 10^5$ cells for each assay.

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