REGULATION OF HERPES SIMPLEX VIRUS-INDUCED THYMIDINE KINASE

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SUMMARY

Thymidine kinase is induced in XC cells infected with herpes simplex type 1 or 2. The messenger for viral thymidine kinase is stable for at least 7 hrs and appears to be an early viral transcript but not a pre-early transcript made in the presence of cycloheximide. Termination of kinase synthesis is dependent upon DNA synthesis. There is a delay of up to 5 hrs between the synthesis of enough mRNA to sustain a maximal rate of kinase synthesis and the increase in detectable kinase activity suggesting that translation is modulated or activation of enzyme is delayed.

INTRODUCTION

Thymidine kinase activity increases after infection of cells with herpes viruses (1-5). The enzyme activity induced after infection has a different pH optimum, Km value, heat sensitivity, drug sensitivity, and antigenic determinants than the normal host isozymes (5-10). Furthermore, experiments utilizing thymidine kinase negative cells (LM TK$^-$) suggest that the herpes virus genome codes for at least part of the induced thymidine kinase activity (2,11,12,13,14).

Regulation of the level of "early" enzyme activities following virus infection has been shown to occur in both bacteriophage and animal virus systems (15-18). This regulation of enzyme activities can be perturbed by blocking viral RNA or viral DNA synthesis in infected cells (15-20). Apart from some studies on the effect of bromodeoxyuridine on regulation of pseudorabies induced thymidine kinase activity (4,20), there has been no thorough study of the regulation of herpes simplex virus (HSV) induced thymidine kinase (TK). Since HSV has been used to transform mouse cells

(25) and XC cells (22) and since there are some contradictory reports on the ability of HSV to induce kinase in XC cells (22,23) we undertook this study of the synthesis and regulation of deoxythymidine kinase in HSV infected XC cells.

In this study we show that: (1) a stable mRNA for thymidine kinase is produced some time before an increase in enzyme activity is observed; the message appears to be an "early" but not a "pre-early" function. (2) thymidine kinase synthesis in HSV infected XC cells is regulated by a "late" function and there is a prolonged delay between the synthesis and expression of messenger RNA.

MATERIALS AND METHODS

Cells and Viruses: XC cells, a Rous Sarcoma Virus transformed rat cell (24) were grown in F-11 (Grand Island Biological Co., N.Y.), supplemented with 10% fetal calf serum, 5mM glutamine, and non-essential amino acids. The macroplaque ($MPdK^-$) strain of type 1 herpes simplex virus (courtesy of Dr. B. Roizman) or a primary isolate of herpes simplex type 2 were used throughout the study. Virus was grown and assayed as previously described (22). Various aspects of the infection of XC cells by HSV have been described previously (22, 23).

Thymidine Kinase: Cultures containing 3.5×10^7 cells were infected at an multiplicity of infection (moi) of 10 and at the indicated times cultures were harvested and assayed for thymidine kinase activity as previously described (22). Kinase specific activity is expressed as CPM of thymidine phosphorylated per 50 µg protein in 15 min. at 36C.

RESULTS AND DISCUSSION

Thymidine kinase was induced in XC cells infected with HSV1 or HSV2. Maximum activity occurred approximately 12-14 hrs. postinfection with HSV1 and 18-20 hrs. postinfection with HSV2 (Fig. 1). After these times, there was a subsequent decrease in enzyme activity.

Previous studies have shown that replication of herpes

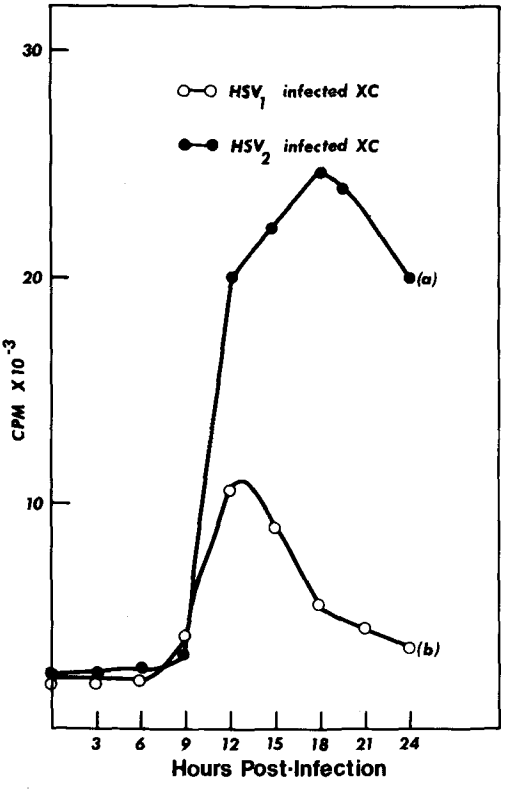

Fig. 1 - Monolayer cultures (75 cm^2) of XC cells were infected with herpes simplex virus type 1 or type 2 at a moi ∿ 10 and cultures were harvested at the indicated times after infection for determination of deoxythymidine kinase activity. Cell extracts were prepared as described in Materials and Methods. In each case 25 µl of cellular extract was assayed for deoxythymidine kinase activity. HSV1 infected cells (o--o) and HSV2 infected XC cells (•--•).

simplex virus is abortive in XC cells but is accompanied by a transient expression of several viral functions (22). The transient expression of thymidine kinase and viral antigens in these cells prompted the following inhibitor studies to examine if thymidine kinase synthesis is controlled in this system. Thymidine kinase synthesis in poxvirus infected cells is controlled at the translational level and if late-viral mRNA synthesis is blocked, no switch-off of thymidine kinase synthesis occurs, leading to a super-induction (18). Studies with another herpes virus, pseudorabies, have shown that thymidine kinase synthesis is arrested at late times in

virus-infected cells and that DNA synthesis is necessary for expression of this control (4, 19-21).

Cultures of XC cells were infected with HSV1 or HSV2 in the presence of cytosine arabinoside or hydroxyurea, inhibitors of DNA synthesis. At the indicated times postinfection, cultures were harvested and lysates assayed for thymidine kinase activity (Figs. 2A, 2B). When DNA synthesis

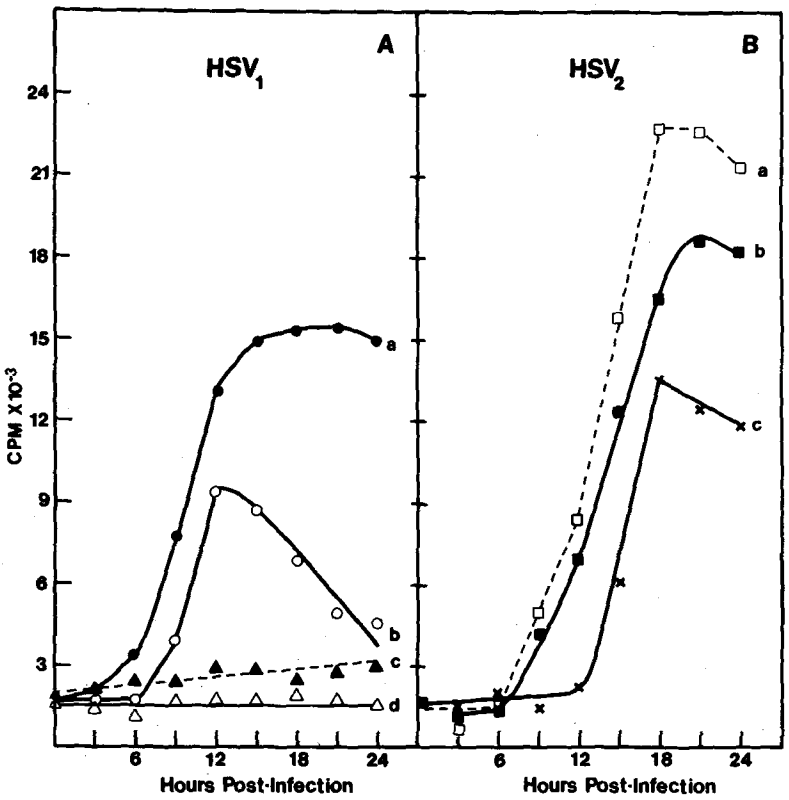

Fig. 2 - Activity of deoxythymidine kinase in extracts of infected XC cells when DNA synthesis is blocked. Fig. 2A - (a) XC cells infected with HSV1 in the presence of 2 x 10^{-4} M hydroxyurea. (b) XC cells infected with HSV1. (c) Mock infected XC cells incubated with 2 x 10^{-4} M hydroxyurea. (d) Mock infected XC cells. Fig. 2B - (a) XC cells infected with HSV2 in the presence of 2 x 10^{-4} M hydroxyurea. (b) XC cells infected with HSV2 in the presence of 2 x 10^{-4} M cytosine arabinoside. (c) XC cells infected with HSV2.

was blocked with these drugs, there was not only a failure of the normal enzyme switch-off mechanism but enzyme activity appeared much earlier than in untreated infected cells (Figs. 2A, 2B).

Since normal regulation of thymidine kinase synthesis is mediated by a late function, it should be possible to block this function with actinomycin D to achieve a super-induction, provided the messenger for kinase is stable. Cultures were infected with HSV1 or HSV2 as described and at 5 or 7 hrs postinfection Actinomycin D (final concentration 2 µg/ml) was added. Cultures were harvested at the indicated times and lysates were assayed for thymidine kinase activity (Figs. 3A, 3B). When

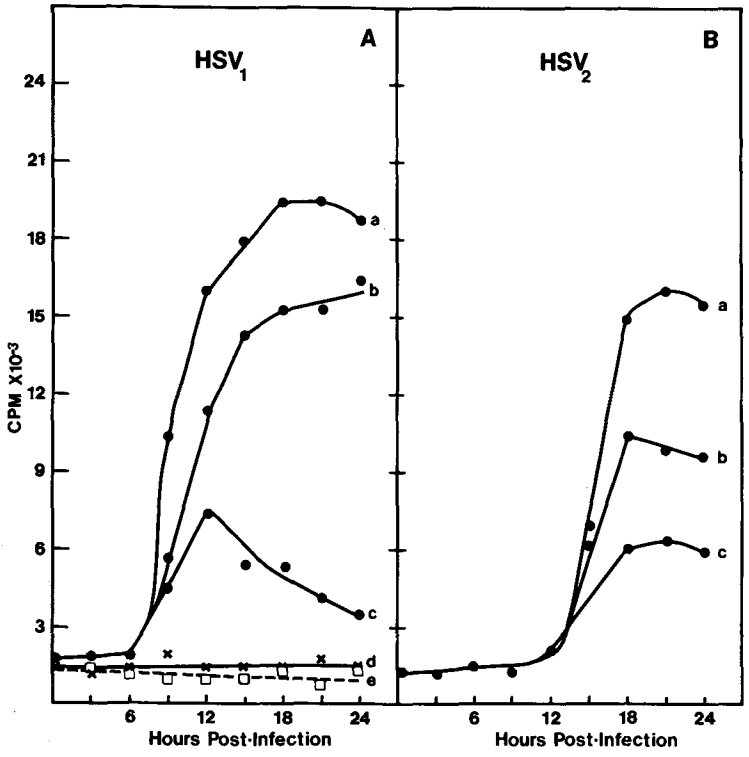

Fig. 3 - Effect of Actinomycin D on the induction of deoxythymidine kinase activity in HSV infected XC cells. 3A - (a) XC cells infected with HSV1 and 2.5 µg Actinomycin D/ml added 7 hrs postinfection. (b) XC cells infected with HSV1 and 2.5 µg Actinomycin D/ml added 5 hrs postinfection. (c) XC cells infected with HSV1. (d) XC cells mock infected and 2.5 µg Actinomycin D/ml added after 5 hrs. (e) Mock infected XC cells. 3B - (a) XC cells infected with HSV2 and 2.5 µg Actinomycin D/ml added 7 hrs postinfection. (b) XC cells infected with HSV2. (c) XC cells infected with HSV2 and 2.5 µg Actinomycin D/ml added 5 hrs postinfection.

actinomycin D was added at 5 hrs postinfection HSV_2 induction of thymidine kinase activity occurred but at a lower level than in normal infection. However, if the drug is added at 7 hrs a super-induction occurs. These data suggest that (1) late mRNA synthesis is necessary for normal regulation of synthesis of thymidine kinase (2) stable mRNA for thymidine kinase is made by 5-7 hrs. postinfection. However, it is noteworthy that an increase in HSV_2 thymidine kinase activity does not occur until 4 to 8 hrs. after mRNA is synthesized. This suggests a control of mRNA translation or delayed activation of enzyme.

All of the above data are consistent with the hypothesis that thymidine kinase is an "early" marker in herpes virus infected cells. Rakusanova *et al*. have shown that in herpes virus infected cells, two classes of early mRNA are made: "early" and (2) "pre-early" (25). The "pre-early" message in contrast to "early" messenger RNA can be synthesized in the presence of cycloheximide when *de novo* protein synthesis is inhibited. Furthermore, in the presence of this drug it is made very rapidly and in larger than normal amounts. Therefore, using the approach of Rakusanova *et al*. it should be possible to ascertain if thymidine kinase mRNA is an "early" or "pre-early" gene. Cultures of XC cells were infected in the presence of cycloheximide for 2 or 4 hrs. At the end of this period the cycloheximide was removed and one set of cultures received Actinomycin D and the other did not. XC cells infected with HSV1 (fig. 4A) show kinase induction if Actinomycin D is not added but no induction is seen if Actinomycin D is added upon removal of cycloheximide. Cultures infected with HSV2 show essentially the same things. Induction is also delayed by 1-2 hrs. in culture treated with cycloheximide perhaps due to a requirement for synthesis of a product which does not occur in the presence of cycloheximide or which is unstable under these conditions. These data show that cycloheximide treatment does not prevent an induction of thymidine kinase by HSV infection if it

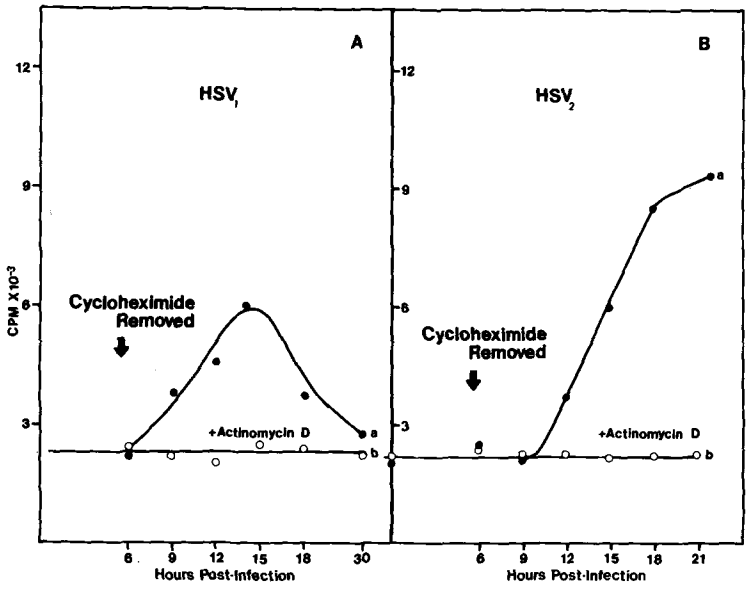

Fig. 4 - Effect of cycloheximide on the synthesis of mRNA for thymidine kinase cultures of XC cells were infected as before in the presence of 50 λ cycloheximide/ml and incubated for 2 hrs at 37C. At this time the cycloheximide was washed out at 4 hrs. 2.5 μg Actinomycin D/ml was then added to one culture in each set and the cultures were re-incubated. The same result was obtained when cycloheximide was removed at 2 hours postinfection. Kinase activity after removal of cycloheximide, no actinomycin D added (•) or after addition of actinomycin D (o).

is washed out and mRNA synthesis is allowed to occur after removal of the drug. However, if new mRNA synthesis is prevented upon removal of the cycloheximide no induction is seen. This suggests that mRNA necessary for the induction of thymidine kinase has not been made during the first 2-4 hours in the presence of cycloheximide. This suggests that thymidine kinase is an "early" but not a "pre-early" gene. The events described above, when examined together with information from direct measurement of rates of synthesis of transcripts (26), should help to pinpoint some of the events involved in replication of herpes viruses.

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