JOURNAL OF VIROLOGY, Mar. 1976, p. 1056-1059
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Vol. 17, No. 3
Printed in U.S.A.

Herpesvirus Proteins: Induction of Nucleoside Phosphotransferase Activity After Herpes Simplex Virus Infection

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Received for publication 6 January 1975

After herpes simplex virus infection of hamster kidney cells there is an induction of nucleoside phosphotransferase activity which can utilize AMP as phosphate donor. The activity is immunologically specific for the infected cell and is induced concomitantly with the virus-coded pyrimidine deoxynucleoside kinase activity. Phosphotransferase activity is not induced in cells lacking both thymidine and deoxycytidine kinase activity.

Phosphorylation of nucleosides may be brought about either by kinases which transfer the γ phosphate group of ATP to the nucleoside, or by nonspecific nucleoside phosphotransferases which catalyze reversible transfer of ester phosphate from nucleoside monophosphate to nucleoside (1).

Examination of extracts prepared from cells infected with herpes simplex virus (HSV) type 1 (6, 7) or 2 (3) has shown that induction of a virus-specific thymidine kinase activity is one of the earliest and most striking events after infection. Recently it has become clear that this thymidine kinase and the virus-induced deoxycytidine kinase activity are due to one enzyme, deoxypyrimidine kinase (4). The two kinase activities cannot be separated on the basis of size or charge (5). In addition, 40 independently isolated mutants of HSV selected specifically for the loss of one kinase always simultaneously lost both activities (4).

By definition, a kinase is an enzyme which requires for its action a nucleoside triphosphate; normally but not exclusively this is ATP, which acts both as the phosphate donor and energy source for the reaction. The investigations of deoxypyrimidine kinase activity were carried out using ATP as phosphate donor. However, there remained the possibility that HSV-infected cell extracts were capable of using other nucleoside phosphates as donors of phosphate in the formation of deoxyribonucleoside monophosphates.

Extracts of HSV-infected BHK cells were prepared as previously described (4) and assayed for ability to phosphorylate thymidine in absence of added ATP but with added ribomonophosphate. In this system, phosphorylating activity could be demonstrated that represented approximately 38% of the ATP-dependent kinase activity on the basis of the rate of phorphorylation. Both AMP and UMP were found to act as donor, while phosphate could be transferred to thymidine, deoxycytidine, and deoxyadenosine (Table 1). In experiments in which ADP was substituted for AMP or where there was no addition of ribonucleotide to the reaction mixture, no significant level of nucleoside phosphorylation was observed (Table 1). Experiments utilizing ^{32}P-labeled AMP suggested that the phosphate transferred arises from the ribomonophosphate and not from a higher phosphate formed during the in vitro reaction (Table 2). No phosphorylation of thymidine is observed if the enzyme is omitted. A higher phosphate acting as donor in these circumstances would have to contain a labeled distal phosphate group; there is no documented series of reactions which would result in the formation of such a molecule from labeled AMP.

The phosphotransferase activity in virus-infected cell extracts is induced in parallel with the deoxypyrimidine kinase activity (Fig. 1) after wild-type virus infection, but induction is not observed after infection with four independent deoxypyrimidine kinase-deficient mutants (a typical example is given in Table 3). Clearly, some virus-coded function is necessary for the induction of phosphotransferase activity. The result implies either that the activities are linked or that the selection system used to isolate deoxypyrimidine kinase-deficient mutants (resistance to BrdUrd or aCyt) has also

TABLE 1. *Phosphotransferase activity of HSV type 1 (strain 17 syn)-infected BHK cell extracts*[a]

Phosphate donor	Nucleoside (nmol) phosphorylated/10 min/mg of protein		
	Substrate		
	Thymidine	Deoxycytidine	Deoxyadenosine
ATP	4.8	2.9	1.8
ADP	0.065	ND[b]	ND
AMP	1.8	1.1	1.2
UMP	2.0	1.42	1.13
None	0.075	0.02	0.01

[a] The reaction mixture contained (final concentration) in a total volume of 100 μl: 5 mM $MgCl_2$, 5 mM AMP, 20 mM phosphate buffer, pH 7, 10 μM substrate, [^{3}H]thymidine, [^{3}H]deoxycytidine or [^{3}H]-deoxyadenosine (10 μCi) and 100 μg of cell extract prepared 8 h after infection as previously described (4). The reaction mixture was incubated at 37 C for 10 min and the products were separated by partition chromatography on paper using a butanol-glacial acetic acid-water (2:1:1, vol/vol) solvent. Where appropriate, 5 mM ADP or 5 mM UMP or 10 mM ATP were used in place of AMP. R_f values of substrates and products of the reactions ATP 5, AMP 15, ADP 9, AdR 70, dAMP 18, dTMP 30, dTTP 8, TdR 80, CdR 75, dCMP 18, dCTP 6.

[b] ND, only examined on one occasion, when no significant phosphorylation was detected.

simultaneously selected for loss of function of an independent gene controlling phosphotransferase activity.

Infection of BHK cells deficient in both thymidine and deoxycytidine kinase activity (PyY/TG/CAR/BUdR) (4) with either wild-type or deoxypyrimidine-kinase-deficient virus fails to induce phosphotransferase activity, although the wild-type virus does induce deoxypyrimidine kinase activity. Infection of cells lacking deoxycytidine kinase alone (PyY/TG/CAR) (4) with wild-type HSV, on the other hand, leads to normal phosphotransferase induction (Table 3).

The extremely low level of phosphotransferase activity which can be demonstrated in uninfected BHK cells (Table 3) is not sensitive to antiserum produced in rabbits against extracts of rabbit cells infected with HSV. However, this same antiserum is found to inhibit the virus-induced phosphotransferase activity to approximately the same extent as the deoxypyrimidine kinase activity, whereas the preimmune serum from the same animal has no effect on either activity (Fig. 2).

These results suggest that after HSV type 1 infection of hamster kidney cells there is induction of a nucleoside phosphotransferase activity which can utilize AMP as phosphate donor and that the activity is immunologically specific for the virus-infected cell. The induction is con-

TABLE 2. *Transfer of phosphate from AMP to TdR*[a]

Compound	Total label added (%)	Counts/min
AMP	98	842,457
ADP	0.0	
ATP	0.0	
dTMP	1.6	13,754[b]
dTDP	0.0	
dTTP	0.0	
Unidentified compound[c]	0.4	3,438

[a] Reaction mixture contained in a total volume of 100 μl (final concentrations): 5 mM $MgCl_2$, 10 μM TdR, 5 mM [^{32}P]AMP, 20 mM phosphate buffer, pH 7, and 100 μg of cell extract prepared 8 h postinfection. The reaction products were separated by partition chromatography using an isobutyric-water-NH_4OH [vol/vol] 66:32:2 solvent. R_f values for substrates and products in this system: AMP, 58; ADP, 46; ATP, 37; TdR, 68; dTMP, 49; dTTP, 26; IMP, 36.

[b] A total of 13,754 counts/min dTMP is equivalent to 0.79 nmol of TdR phosphorylated/mg of protein.

[c] This compound ran coincidentally with phosphate during the partition chromatography, but was not further investigated.

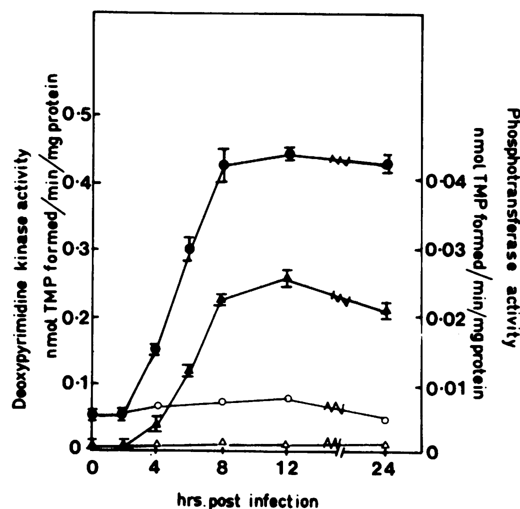

FIG. 1. *Induction of deoxypyrimidine kinase and phosphotransferase activity (measured as described in the legend to Table 1) after wild-type HSV type 1 (strain 17 syn) infection of BHK C13 cells. Activity expressed as nanomoles TMP formed per minute per milligram of protein. Symbols: (●) HSV deoxyprimidine kinase; (▲) HSV phosphotransferase; (O) uninfected BHK C13 cell thymidine kinase; (Δ) uninfected BHK C13 cell phosphotransferase.*

TABLE 3. *ATP- and AMP-mediated thymidine phosphorylation by herpes simplex-infected cell extracts*[a]

Cells	Virus	TMP (nmol) formed/ 10 min/mg of protein	
		ATP mediated	AMP mediated
BHK C13		1.64	0.13
BHK C13	17 syn	3.64	0.45
BHK C13	17 dPyK-[b]	0.73	0.06
PyY/TG/CAR BUdR[c]		0.12	0
PyY/TG/CAR/ BUdR	17 syn	5.83	0.01
PyY/TG/CAR/ BUdR	17 dPyK-	0.08	0
PyY/TG/CAR[d]		3.19	0.08
PyY/TG/CAR	17 syn	5.72	0.56
PyY/TG/CAR	17 dPyK-	2.51	0.07

[a] See Footnote *a*, Table 1.

[b] Lacking deoxypyrimidine kinase activity.

[c] Lacking thymidine and deoxycytidine kinase activity (and also HGPRTase activity).

[d] Lacking deoxycytidine kinase activity (and also HGPRTase activity).

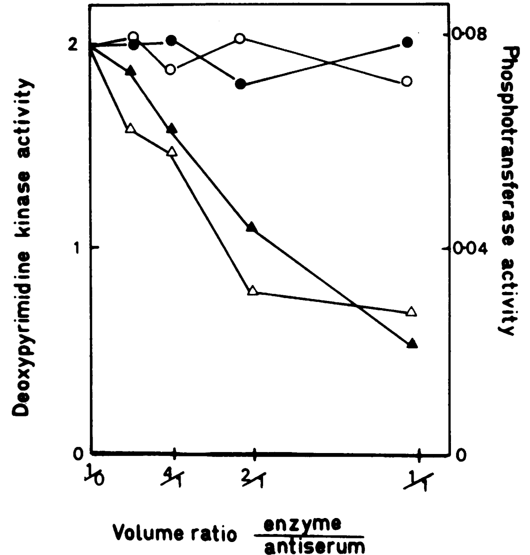

FIG. 2. *Effect of preimmune serum and HSV-type 1 (strain 17 syn) specific antiserum on HSV-1-induced deoxypyrimidine kinase and phosphotransferase activities. Aliquots of enzyme extracts were preincubated for 10 min at 4 C with an equal volume of antiserum at the appropriate dilution and then assayed as described in footnote a, Table 1. Activity is expressed as nanomoles of TMP formed per minute per milligram of protein. Symbols: (●) HSV-induced deoxypyrimidine kinase plus preimmune serum; (○) HSV-induced phosphotransferase plus preimmune serum; (▲) HSV-induced deoxypyrimidine kinase plus HSV antiserum; (△) HSV-induced phosphotranfrase plus HSV antiserum.*

comitant with that of deoxypyrimidine kinase activity. Successful phosphotransferase induction, however, depends both on the ability of the virus genome to induce deoxypyrimidine kinase and on a cell genome-specified function which is lacking in the BrdUrd-resistant cells (but not in the aCyt-resistant cells from which the PyY/TG/CAR/BUdR line was derived). Less extensive investigations in our laboratory with HSV type 2 have given identical results, and antisera produced against HSV type 2-infected cell extracts inhibit both type 1- and type 2-induced phosphotransferase activities. This mimics the pattern of inhibition observed with thymidine kinase activity.

The phosphotransferase activity of infected cells is rather unstable, and although attempts have been made physically to separate kinase and phosphotransferase, loss of activity on handling presents problems for quantitative assessment and purification.

It is clear that no hypothesis which depends exclusively on virus specification or on cell specification for the induction of phosphotransferase activity in the HSV-infected cell could accommodate the data of both Fig. 2 and Table 3. However, at least two mechanisms of induction could explain all the data.

First, nucleoside phosphotransferase activity may constitute a further potential function of the virus-induced deoxypyrimidine kinase, but require for activity or in vitro stability, an internal cell environment present in BrdUrd-sensitive but absent from BrdUrd-resistant cells. Second, phosphotransferase activity in the infected cell may result from an interaction between viral and cellular polypeptides normally involved in the respective thymidine kinase activities. Our data on the sedimentation characteristics of the viral deoxypyrimidine kinase (4) indicates that this enzyme probably exists in an aggregated form. Thus it is not inconceivable that some cell polypeptide can participate in such an aggregate.

The hypothesis that induction of phosphotransferase activity could be due to derepression of a cellular enzyme and that BrdUrd-resistant cells have been selected for lack of this function is not compatible with the data in Fig. 2.

The role of nucleoside phosphotransferase

activity in the presence of a quantitatively much more active deoxypyrimidine kinase activity is difficult to assess (2), but in natural infection, all phosphate scavenging ability may be at a premium in the herpes-infected cell where the demand for phosphorylated deoxyribonucleosides is high and extended. It is clear that the relative pool sizes of deoxynucleotides undergo considerable change after herpesvirus infection (A. T. Jamieson and G. Bjursell, J. Gen. Virol., in press), and a possible function of phosphotransferase may be the movement of phosphate to preserve balance.

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