EFFECT OF SPERMINE ON THE ACTIVITY OF HERPES SIMPLEX VIRUS TYPE 1 DNA POLYMERASE

Influence of the template

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1. Introduction

The polyamines, putrescine, spermidine and spermine are cellular polycations whose intracellular concentrations appear to be important in the regulation of macromolecular synthesis and growth of many types of cells and viruses [1,2]. Putrescine and the related polyamines have been shown to have marked growth-stimulating activity in several cell systems including chinese hamster cells [3] and human embryonic fibroblasts [4]. Similarly, some DNA viruses such as vaccinia, human cytomegalovirus and herpes simplex virus (HSV) have an obligate requirement for polyamines for virus production [5–7].

The site of action of the polyamines in cells and viruses is not known, although they may be necessary for DNA replication [8,9]. For example, treatment of lymphocytes with methylglyoxal bis(guanylhydrazone) or α-methylornithine, both of which inhibit the biosynthesis of polyamines, resulted in specific inhibition of DNA synthesis. Since neither drug had any effect on DNA synthesis in vitro, and since the degree of inhibition of DNA synthesis correlated with the degree of polyamine deficiency, it was concluded that DNA replication was one cellular site of action of the polyamines [9]. Similarly in work with viruses evidence from studies in vitro has shown that polyamines optimise the synthesis of HSV-DNA [10] and that both spermidine and spermine stimulate the activity of purified HSV-1 DNA polymerase [11].

Here, we have examined the influence of the template-primer on the stimulation of HSV-1 DNA polymerase by spermine. The results show that stimulation is critically dependent on the presence of a natural DNA template-primer and on the ratio of that DNA to spermine. Thus spermine stimulates the virus polymerase in a manner which suggests interaction with the template rather than with the enzyme.

2. Materials and methods

BHK-21/C13 cells and HSV-1, strain 17, were grown as in [12]. HSV-1 DNA polymerase was purified from infected monolayers of BHK-21/C13 cells 16 h after infection [11]. The polymerase was purified 545-fold by this method.

The assay for the processiveness of HSV-1 DNA polymerase contained, in a final volume of 200 μl, 100 mM Tris–HCl (pH 8.2) at 39°C, 5 mM dithiothreitol, 3 mM $MgCl_2$, 25 μM d[^{3}H]GTP (0.25 Ci/mmol), 20 μg poly(dC), 1 μg oligo$(dG)_{12-18}$ and 0.26 μg enzyme preparation. The assay was incubated at 39°C for 15 min and was terminated by the addition of ethylenediaminetetracetic acid to 20 mM final conc.; the amount of synthesis was determined by DE-81 paper assay [13]. The reaction mixture was loaded onto a 0.5 ml column of oligo(dG)-cellulose which was then washed with 9 × 1 ml portions of 0.2 M NaCl. The reaction product was digested for 1 h at 37°C with micrococcal nuclease, then for 3 h with spleen phosphodiesterase [14]. Samples (20 μl) were

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applied to DE-81 paper and the products of digestion, [^{3}H]GdR and 3′-d[^{3}H]GMP, separated by ascending chromatography with 3 mM ammonium formate (pH 3.6) as solvent. Appropriate areas of the paper were cut out and soaked in 0.6 ml of 0.5 M NaCl in 1 M HCl to elute [^{3}H]GdR and d[^{3}H]GMP, which were counted for radioactivity in a Triton–toluene-based scintillation fluid.

The method was the same when poly(dA) · oligo(dT) was used as the template-primer except that derivatives of thymidine and deoxyadenosine replaced the corresponding derivatives of deoxyguanosine and deoxycytidine.

3. Results and discussion

As observed in [11] spermine stimulated the activity of purified HSV-1 DNA polymerase (fig.1). However, the extent of this stimulation depended on the concentration of polyamine and also on the amount of activated DNA used in the assay. Using activated DNA at 20 μg/assay spermine always stimulated the reaction, maximal stimulation being 3-fold at 2.5 mM spermine (fig.1), which is equivalent to a ratio (μg activated DNA:μg spermine) of 0.11. When the amount of activated DNA in the assay was decreased both the extent of maximal stimulation by spermine, and the polyamine concentration required to achieve this, also decreased (fig.1). The DNA:polyamine ratio required to achieve maximal stimulation therefore remained constant at ~0.1. Thus it appeared that this ratio, and not the concentrations of polyamine or DNA, was the important factor in determining the extent of stimulation of the reaction by spermine.

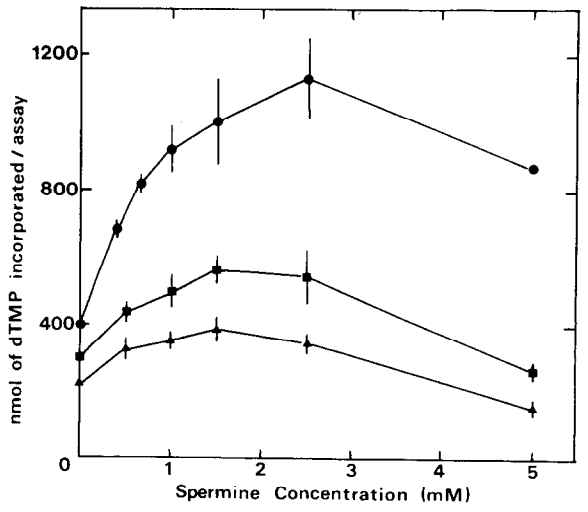

Fig.1. The effect of various amounts of activated DNA on the stimulation of HSV-1 DNA polymerase by spermine. The reaction was done as in [11] using 5 μg (▲), 10 μg (■) or 20 μg (●) of activated DNA/assay. Spermine was added to the assay mixture immediately before addition of the enzyme. Values are means ± SD (n = 3).

Since stimulation depended on the concentration of the template-primer it seemed likely that spermine influenced the activity of the HSV-1 DNA polymerase indirectly by interacting with the DNA. Stimulation by the polyamine could be caused by either an increase in the number of initiation sites available to the enzyme and/or by an increase in the extent of polynucleotide chain elongation. We attempted to distinguish between these two possibilities using synthetic template-primer DNA molecules.

When poly(dA) · oligo(dT) and poly(dC) · oligo(dG) were used in the assay spermine severely inhibited the activity of the HSV-1 DNA polymerase (table 1), in contrast to the stimulation observed using activated DNA. The synthetic template-primers were constructed such that the primers, oligo(dT) and oligo(dG), were present at saturating levels with respect to the polymerase. Therefore, the maximum number of initiation sites was available at all times. The observed inhibition of the polymerase activity by spermine presumably then resulted as a consequence of the polyamine preventing the utilisation of initiation sites, or preventing movement of polymerase along the DNA. Processiveness assays enabled us to distinguish between these alternative explanations.

Processiveness was estimated by measuring the ability of the polymerase to incorporate radioisotopically-labelled nucleotides covalently into a primed DNA template. For example, using poly(dC) · oligo(dG) processiveness was determined by measuring the incorporation of d[^{3}H]GMP into an oligo(dG) primer hydrogen bonded to a poly(dC) template. The chain length of the DNA synthesised in the assay was then calculated from the equation:

$$\text{Chain length} = \frac{\text{cpm in GdR} + \text{cpm in 3′-dGMP}}{\text{cpm in GdR}}$$

where GdR was derived from the 3′-terminus of the polynucleotide chains and dGMP was from the internal residues of the chains after digestion with nuclease. In common with calf thymus DNA polymerases

Table 1
The effect of spermine on the activity of HSV-1 DNA polymerase (nmol ^{3}H-labelled nucleotide incorporated/assay) in the presence of various templates

Template	Spermine (mM)		
	0	1.0	5.0
Activated DNA	301 ± 20	500 ± 69	253 ± 7
Poly(dA–dT) · poly(dA–dT)	225 ± 23	97 ± 17	49 ± 7
Poly(dC) · oligo(dG)$_{12-18}$	1816 ± 37	75 ± 3	10 ± 5
Poly(dA) · oligo(dT)$_{12-18}$	2799 ± 63	2829 ± 80	177 ± 12

The assays were done as in section 7 using the templates indicated at 10.5 μg/assay. In addition each assay contained spermine at the level indicated. Values are means ± SD (n = 3)

[17] HSV-1 DNA polymerase was quasi-processive (table 2). In the absence of polyamine polynucleotide chains synthesised using poly(dC) · oligo(dG) were 4-times the length of those synthesised using poly(dA) · oligo(dT). Since HSV-1 DNA has a high (G + C) content (67%) it seems reasonable to propose that the virus DNA polymerase is more efficient at replicating a template rich in guanosine and cytosine. The inhibitory effect of spermine on the virus polymerase using poly(dC) · oligo(dG) was clearly on the elongation of the polynucleotide chains (table 2). In contrast when poly(dA) · oligo(dT) was the template-primer spermine appeared to inhibit chain initiation,

Table 2
The effect of spermine on the processiveness of HSV-1 DNA polymerase

Spermine (mM)	Chain length	^{3}H-Labelled nucleotide incorp. (% nmol in the absence of spermine)
Poly(dC) · oligo(dG)$_{12-18}$[a]		
0	20.1 ± 1.2	100
1.0	1.9 ± 0.1	4
5.0	3.5 ± 0.9	1
Poly(dA) · oligo(dT)$_{12-18}$[b]		
0	5.0 ± 0.3	100
0.75	4.9 ± 0.4	90
1.5	4.0 ± 0.2	41

100% incorporation is equivalent to [a]1816 ± 37 and [b]2799 ± 63 of ^{3}H-labelled nucleotide incorporated/assay

The chain length of oligo(dG) or oligo(dT) was measured as in section 2. Each assay also contained spermine in the concentration indicated. Values are means ± SD (n = 3)

since there was no measurable decrease in the average length of polynucleotide chains synthesised despite a 60% decrease in polymerase activity (table 2). It is possible however in this case, in view of the difficulties of measuring differences in relatively short chain lengths (4–5 units), that some diminution of chain lengths could have gone undetected.

The inhibition of polymerase activity using these synthetic template-primers contrasts with the stimulation observed when activated DNA is used. It is possible that spermine destabilises the structure of activated DNA, thus stimulating the reaction in a manner similar to that of an unwinding protein [15]. Interaction with the synthetic DNA molecules, on the other hand, may result in their condensation with consequent effects on the initiation and/or elongation of polynucleotide chains as discussed above.

In conclusion, we have shown that stimulation of HSV-1 DNA polymerase by spermine clearly depends both on the nature of the DNA used as the template-primer, and on the ratio of the template-primer to polyamine and in the assay. Therefore, the concentration of polyamine alone is not sufficient to define reaction conditions. In the light of these new observations some of the contradictory findings previously reported on the effects of polyamines on DNA polymerase (see [16]) may now be understood.

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