Temperature-sensitive Mutants in Two Distinct Complementation Groups of Herpes Simplex Virus Type 1 Specify Thermolabile DNA Polymerase

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SUMMARY

We and others have previously shown that a mutant in herpes simplex virus type 1 (HSV-1) complementation group 1-4 induces a virus-specified DNA polymerase that is temperature-sensitive in vitro. Here we report that HSV-1 tsC7 in complementation group 1-3 also induces a thermolabile DNA polymerase.

Herpes simplex virus type 1 (HSV-1) induces a DNA polymerase which consists of a polypeptide of about 150000 mol. wt. (Powell & Purifoy, 1977). The enzyme appears to be active, at least in vitro, as a monomer (see Discussion below). At least two enzymic activities reside in the purified enzyme, the DNA polymerase and an associated nuclease (Weissbach et al., 1973, Knopf, 1979). Temperature-sensitive (ts) mutants in two separate complementation groups of HSV-1 (1-3 and 1-4) induce an enzyme which is apparently temperature-sensitive in vivo, as measured by monitoring the polymerase activity in infected cells following a shift to non-permissive temperature (Aron et al., 1975). Only one mutant so far, HSV-1 tsD9 (complementation group 1-4), has been shown to induce an enzyme which is thermolabile in vitro, i.e. the purified enzyme exhibits a temperature-sensitive phenotype (Purifoy et al., 1977). Obviously in in vivo temperature-shift experiments the DNA polymerase can be severely affected by other proteins in the DNA replication complex. The virus DNA polymerase gene and the mutations of tsD9 and tsC7 (complementation group 1-3) have been mapped on the genetic and physical maps of HSV-1 DNA by genetic recombination using three-factor crosses and by using intertypic recombinants. The mutations for phosphonoacetic acid (PAA) resistance, and for tsD9 and tsC7 all map very closely together both genetically and physically (Chartrand et al., 1979, 1980; Purifoy & Powell, 1977). More recently, one of the loci specifying acycloguanosine resistance has also been mapped to the same area of the genome (Crumpacker et al., 1980). Since mutants in groups 1-3 (tsC7) and 1-4 (tsD9) complement each other (Schaffer et al., 1973) and yet they apparently map extremely closely together, it was of interest to determine if the mutant in group 1-3 (tsC7) induced a thermolabile polymerase. This was particularly the case since the region of the genome where tsC7 maps is located to the left of that of tsD9, so that tsC7 might be suspected of being in a gene specifying a separate protein. The virus-specific DNA polymerase from tsC7-infected cells was prepared as detailed previously (Powell & Purifoy, 1977). Briefly, cells were extracted with high salt and the extract chromatographed on DEAE-cellulose. The fractions containing DNA polymerase activity were located, pooled, and the pool dialysed against a suitable buffer. This procedure was repeated through chromatography on phosphocellulose and DNA cellulose. The final peak of DNA polymerase from DNA cellulose was used for further experiments. The purification of the mutant enzyme is shown in Fig. 1. The temperature stability of the enzyme derived from tsC7-infected cells was tested (shown in Fig. 2a) by heating the enzyme in reaction mixture before adding DNA template (Purifoy et al., 1977). From this result it was clear that the enzyme in tsC7 was thermolabile compared to the wild-type strain (KOS) enzyme. The enzyme from tsC7- and tsD9-infected cells was then compared (Fig. 2b). Both enzymes were
Fig. 1. Purification of HSV-1 tsC7 DNA polymerase. After high salt extraction the DNA polymerase was purified by passage through (a) DEAE-cellulose, (b) phosphocellulose and (c) DNA cellulose. The level of DNA polymerase (●) was monitored by our standard assay (Powell & Purifoy 1977) and salt concentration determined by refractometry. The pure enzyme as recovered from the DNA cellulose column was used in all the experiments.

Fig. 2. Heat inactivation of wild-type and mutant enzymes. DNA polymerase purified from cells infected with wild-type (O), tsC7 (●) or tsD9 (○) was heated at 39 °C in reaction mixture; template DNA was then added and the residual DNA polymerase activity determined using standard assay methods. The specific activity of the enzymes was between $4 \times 10^3$ to $5 \times 10^3$ units/mg. One-hundred % activity corresponds to 140 000 ct/min in the assay of each enzyme.

thermolabile but the tsD9 enzyme appeared more sensitive to heating than did the tsC7 enzyme.

The results indicate that mutants in complementation groups 1-3 and 1-4 of HSV-1 induce polymerase molecules bearing a temperature-sensitive defect. It is relevant, therefore, to discuss the polypeptide constituents of purified DNA polymerase. Our preparations contained one major virus-specific polypeptide of 150 000 mol. wt. with a minor contaminant, which was found in varying amounts up to 1/3 of the major protein by molar ratio and did not possess polymerase activity alone (Powell & Purifoy, 1977). Allen et al. (1977) found a single polypeptide of 160 000 mol. wt. in purified preparations of equine abortion virus DNA polymerase. Knopf (1979) found in his preparations of enzyme, eight to ten polypeptides of which three were associated with DNA polymerase activity: these three proteins were of 144 000, 74 000 and 29 000 mol. wt. These results indicate that the major polypeptide associated with DNA polymerase is of about 150 000 mol. wt. and that other smaller proteins can be associated with this polypeptide. Perhaps more pertinent, studies of the active size of the enzyme estimated by sedimentation in glycerol or sucrose gradients all agree that the active size of the enzyme in vitro is 120 000 to 160 000 mol. wt. (Allen et al., 1977; Knopf,
1979; Ostrander & Cheng, 1980; K. L. Powell & D. J. M. Purifoy, unpublished observations). Thus, in vitro purified DNA polymerase appears to be a single polypeptide enzyme active as a monomer, but this may not be the functional unit in the infected cell.

We have confirmed the complementation for infectious virus in quantitative yield tests described by Schaffer et al. (1973) for tsC4 and tsC7 with tsD9, although the complementation indices we obtained were lower (5 and 8, respectively) than previously reported. Since tsC7 and tsD9 both induce polymerase molecules that are thermolabile, this complementation may be intragenic. The results presented here and those of Honess & Watson (1977) on PAA-sensitive and -resistant mutants in mixed infection indicate that the DNA replication complex of the virus may contain two or more polymerase molecules which can effect complementation. In a recent study tsH, an HSV-1 ts mutant defective in DNA polymerase, has been shown to fail to complement either tsC7 or tsD9, a pattern which might be expected if the complementation observed between C and D group mutants is intragenic (Chartrand et al., 1980). A less attractive explanation would be that tsH contains two ts lesions, one in the gene product represented by tsC7 and one in that represented by tsD9. Intracistronic complementation between two ts mutants is difficult to prove; an analysis of ts+ revertants of tsH, tsC4 and tsC7 might be helpful.

Recently, a number of mutations associated with virus DNA polymerase have been elegantly mapped by Chartrand et al. (1979, 1980); all cluster in a 4·9 kilobase pair (kbp) region between 38·6 and 41·8 map units. Most of these mutations, including those for PAA resistance, for tsD9 and tsH map in the right hand subset of this region (39·6 to 41·8 map units), whereas that of tsC7 maps just to the left of this region (38·6 to 39·6 map units) and that of an additional mutant in group 1-3 tsC4 maps between 39·3 and 41·8 map units (Chartrand et al., 1980). These workers speculated that should the 4·9 kbp region contain two separate genes, it was likely that the mutation of tsC7 was in one and those of tsD9, tsH and PAA resistance in the other. Our data clearly show that the mutation in tsC7 results in a thermolabile DNA polymerase; thus if two gene products are represented they should both be components of the purified polymerase.

Parris et al. (1978) claimed to distinguish the mutations of tsC4, which exhibited a transcriptional block in their study, from that of tsD9 which did not. However, the effects of actinomycin D observed with tsC4 and tsD9 may be solely quantitative rather than a qualitative difference in the effects of the two ts mutations represented (see Parris et al., 1978, Fig. 2 and Table 1).

To date, some 23 complementation groups of HSV-1 mutants have been established (Schaffer et al., 1978). Mutants in more than half of these groups are defective in virus DNA synthesis, and those in at least five separate complementation groups are affected in DNA polymerase induction (Aron et al., 1975). Clearly, with mutants in group 1-2, such as tsB2, the reduction in polymerase is due to altered α protein function decreasing the production of β proteins including the DNA polymerase (Honess & Roizman, 1974). The results presented here may suggest that other mutants deficient in polymerase induction may have bona fide lesions in the enzyme if the complementation observed is intragenic. The number of essential cistrons represented by the current HSV-1 complementation groups may thus be fewer than was previously thought. Obviously the purified enzyme from other mutants should be examined to clarify this point.

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