DIFFERENTIAL HEAT SENSITIVITY OF MAMMALIAN DNA POLYMERASES

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SUMMARY: DNA polymerase-β from a variety of mammalian cells is more rapidly inactivated in vitro by elevated temperature than DNA polymerase-α. This differential thermolability is observed between 40 and 50°C and also occurs in the presence of added DNA. It is suggested that heat sensitivity provides a new criterion for delineating DNA polymerase-β and a possible means of examining the function of this enzyme in vivo.

Mammalian cells have been shown to contain multiple species of DNA polymerase (1-3). These have been designated as DNA polymerase-α, β, γ, and mitochondrial (mt). The enzymes are commonly distinguished from each other on the basis of size, inhibition by sulfhydryl reagents, template utilization, and intracellular localization (1-4). DNA polymerases α and β preferentially utilize activated DNA as a template while DNA polymerase-γ exhibits an unusual propensity for copying poly (rA).oligo (dT). The function of DNA polymerases in the cellular metabolism of bacteria has been rigorously defined only by the use of conditional mutants. At present, such mutant mammalian cells are not available. However, the increase in DNA polymerase-α upon the initiation of DNA replication in resting cells suggests that DNA polymerase-α functions in DNA replication (1-3). It has been proposed that DNA polymerase-β functions in DNA repair (1, 5). We now report that DNA polymerase-β isolated from a variety of mammalian cells is heat sensitive. This thermolability provides an additional criterion for characterizing this class of polymerase and an experimental probe for establishing its physiological function.

The kinetics of heat inactivation of DNA polymerase-α, and -β from a variety of mammalian sources are shown in Fig. 1. In each experiment partially purified preparations of DNA polymerase-α and -β were simultaneously

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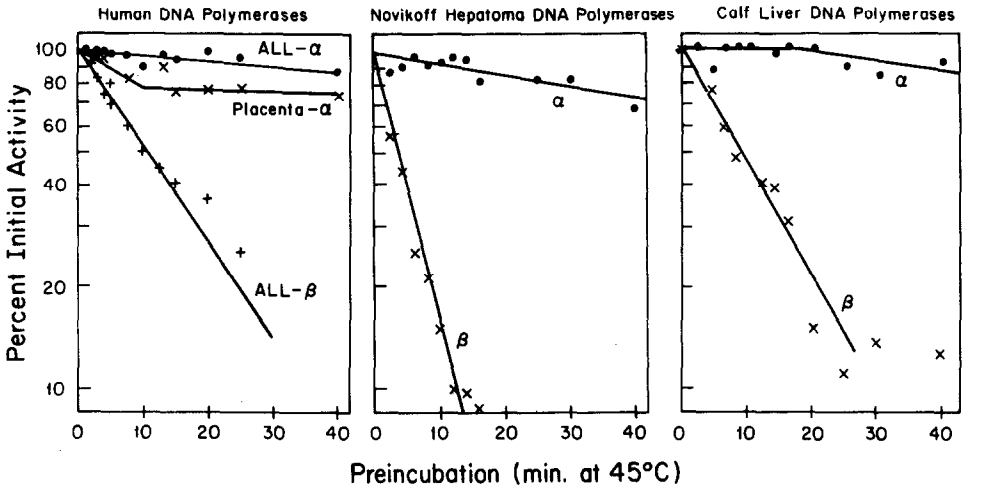

Fig. 1: Heat inactivation of DNA polymerase-α and β. DNA polymerase-α and β were purified from acute lymphatic leukemia cells by a modification of the method of Lewis et al. (8). This involved homogenization of lymphocytes in high salt, separation of DNA polymerase-α from -β by chromatography on DEAE and purification of the separated enzymes by chromatography on phosphocellulose (Dube, in preparation). DNA polymerase-α was purified from human placenta by a similar procedure involving additional chromatography on Sephadex G-100 (Seal, in preparation). The partially purified DNA polymerase-α and highly purified DNA polymerase-β (fraction 6) from Novikoff hepatoma cells was a generous gift of R. Meyer (University of Cincinnati). The calf thymus DNA polymerase-α was a generous gift of J. Furth (University of Pennsylvania) and calf thymus DNA polymerase-β was generously provided by L. Chang (University of Connecticut). Heat inactivation of different DNA polymerases was carried out as follows: for each sample tested, an 0.5 ml aliquot containing 0.5 to 1.0 unit of DNA polymerase was adjusted to 1 mg/ml of protein with bovine serum albumin, 10 mM Tris-HCl (pH 7.8) and 0.01 M KCl. The mixture was incubated at 45°. At the times indicated, 20 µl aliqouts were removed and assayed for DNA polymerase activity.

DNA polymerase assays (0.1 ml) were carried out in duplicate and contained: 25 mM Tris-HCl (pH 8.4), 5 mM 2-mercaptoethanol, 10 mM $MgCl_2$, 50 mM KCl, 100 µM each of dATP, dGTP, dCTP and (α-^{32}P) dTTP (100-200 cpm/pmole), 100 µg/ml activated DNA, 15% (v/v) glycerol, 10 µg bovine serum albumin and 0.1-0.2 unit of enzyme. Incubation was carried out for 60 min at 37°C. The reactions were stopped by the addition of perchloric acid and the acid insoluble precipitate was collected for determination of radioactivity as previously described (9). Initial activity (100%) corresponds to 50-75 pmoles dTMP in each experiment.

preincubated at 45°C for the times indicated and the remaining activity was determined at 37°C using activated DNA as a template. DNA polymerase-β activity declined 50% within 5-10 min while DNA polymerase-α activity was not significantly reduced. None of the DNA polymerase-α

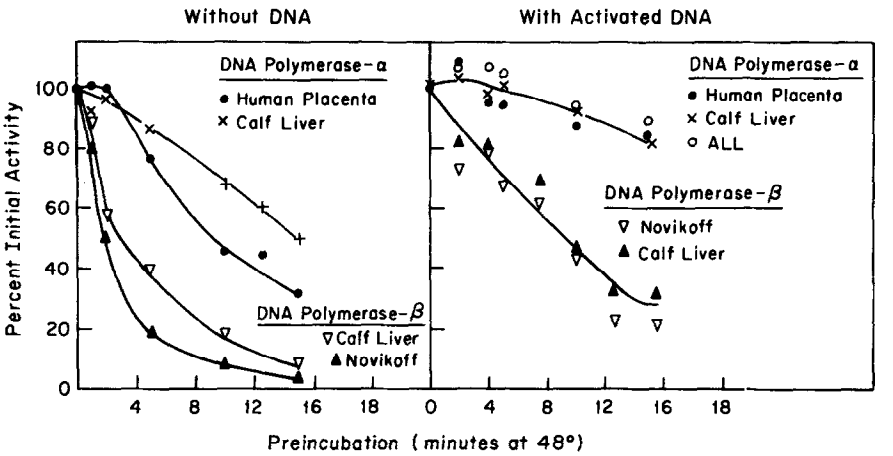

Fig. 2: Effect of Activated Calf Thymus DNA on Heat Sensitivity. Experimental conditions are as described in the legend to Fig. 1. Preincubation was carried out at 48°C with (2A) and without (2B) added activated DNA (100 μg/ml). Activated DNA was prepared as previously described by treatment of calf thymus DNA with DNase until maximum priming activity was achieved for sea urchin nuclear DNA polymerase. The average molecular weight of the hydrolyzed DNA corresponds to 70,000 (10).

preparations show a heat sensitive component except the preparation from human placenta which appears to contain a small amount of a heat sensitive DNA polymerase, presumably DNA polymerase-β.

DNA polymerase-β is usually found in nuclei isolated from eucaryotic cells. It has a high affinity for DNA (1) and, in fact, can be separated from DNA polymerase-α by affinity chromatography (6). The possibility that the thermolability of DNA polymerase-β might be due to a bound DNA contaminant was examined by testing the effect of added activated DNA on the rate of polymerase inactivation (Fig. 2). At 48°C the added DNA protects both DNA polymerase-α and -β against heat inactivation and therefore does not account for the thermolability of DNA polymerase-β. At 50°C the added DNA protects only DNA polymerase-α (results not shown). Thus, inactivation in the presence of DNA at 50°C might be an even more exacting criterion for discriminating between α and β DNA polymerase.

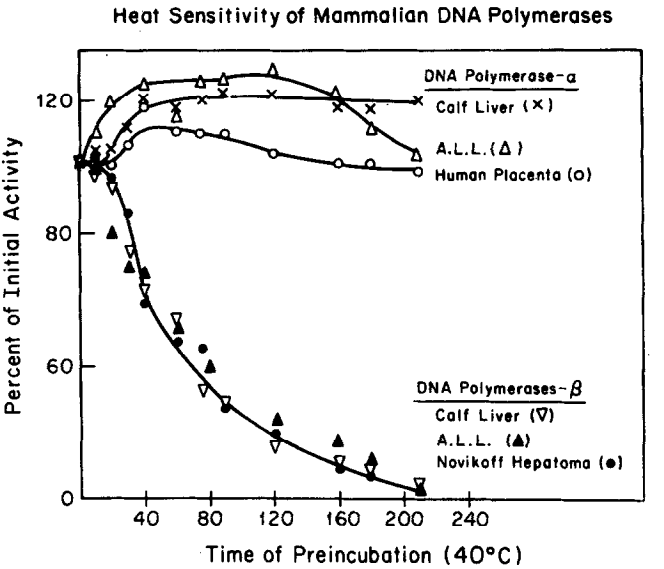

Fig. 3: Heat Inactivation at 40°C. Experimental details are those given in the legend to Fig. 1 except that preincubation was carried out at 40°C in presence of activated calf thymus DNA (100 μg/ml).

The kinetics of heat inactivation of DNA polymerase α and β were also studied at 40°C in the presence of activated DNA (Fig. 3). At this temperature the $T_{1/2}$ for DNA polymerase-β from three mammalian sources is 40-60 min while no loss of activity of DNA polymerase-α is observed for as long as 210 min. In initial experiments, exposure of mouse L-cells to 40°C for 6 h did not result in the inactivation of DNA polymerase-β. Higher temperature or longer time may be required for inactivation of DNA polymerase-β which is tightly bound to chromatin in cells.

The observations in this paper on the heat sensitivity of DNA polymerase-β from human, calf and rat cells provide an additional criterion for delineating this class of DNA polymerases. DNA polymerase-β is usually defined on the basis of its size in sucrose gradients as well as its insensitivity to sulfhydryl reagents such as N-ethyl maleimide.

Size determination by sedimentation, may be an unreliable criterion due to the possible formation of aggregates, particularly in crude extracts. Differential inhibition by N-ethyl maleimide may be inadequate since Mosbaugh et al. (7) reported that the DNA polymerase-β from Novikoff hepatoma is partially sensitive to N-ethyl maleimide. We have confirmed these results using DNA polymerase-β from a variety of other sources (unpublished data).

The sensitivity of DNA polymerase-β to heat is in contrast to the relative resistance of this enzyme to denaturation by urea and ethanol (1). Analysis of the rates of DNA replication and repair in cells and in isolated nuclei at different temperatures may provide insight into the role of DNA polymerase-β in these processes.

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