Differential effects of dimethylsulfoxide on the activities of human DNA polymerases α and δ

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ABSTRACT

The effects of dimethylsulfoxide on the activities of purified human placental DNA polymerase α and DNA polymerase δ were examined. DNA polymerase α was inhibited by dimethylsulfoxide, whereas DNA polymerase δ was significantly activated, by as much as 6-fold. Kinetic data show that the effect of dimethylsulfoxide on DNA polymerase δ activity was due to a reduction in the apparent K_m for its substrate, dTTP. This novel finding of the differential effects of dimethylsulfoxide on the activities of polymerases α and δ may be useful in their identification and differential assay.

INTRODUCTION

Mammalian DNA polymerases can be classified as being of four types, α , β , γ and δ (1). DNA polymerase α has been implicated as being involved in DNA replication, based on evidence from studies of inhibitors of DNA synthesis, particularly by aphidicolin, which inhibits DNA synthesis *in vivo* and also selectively inhibits polymerase α activity (but not β and γ) *in vitro* (1,2). This evidence is also supported by the nuclear localization of DNA polymerase α (3) and the inhibition of DNA synthesis in permeabilized cells by anti-polymerase α antibodies (4). DNA polymerase δ , a more recently described enzyme (5-9) is unlike polymerase α in that it possesses an associated and apparently intrinsic 3' to 5' exonuclease activity which is not separated from the polymerase activity by any of the chromatographic and analytical procedures so far used. The significance of the 3' to 5' exonuclease activity is that it has the potential to provide a proof-reading or editing function. Like polymerase α , DNA polymerase δ is also inhibited by aphidicolin (8,9). The issue of whether polymerase δ is a distinct enzyme from α remains to be resolved, since there have also been reports of the isolation of polymerase α forms which have associated 3' to 5' exonuclease activities (10,11). We report here a novel effect of dimethylsulfoxide (DMSO) on DNA polymerase δ activity, which is apparently activated by this solvent, whereas DNA polymerase α is inhibited. These effects

provide a simple means for the differentiation of polymerase δ from DNA polymerase α activity.

METHODS AND MATERIALS.

Materials. Poly(dA-dI) and *E. coli* Pol I were obtained from Boehringer-Mannheim. DMSO was purchased from Mallinckrodt. BuAdATP [2-(*p*-n-butylanilino)-deoxyATP] was a generous gift from Dr G.E. Wright, University of Massachusetts Medical School, Worcester, MA.

Enzymes. Human placental DNA polymerase δ and α were prepared as described elsewhere (9). The polymerase δ preparation had a specific activity of 48,000 units/mg using poly(dA-dT) as the template-primer, and the polymerase α preparation had a specific activity of 25,000 units/mg assayed with activated calf thymus DNA. The polymerase α preparation was devoid of exonuclease activity.

Enzyme assays. DNA polymerase activities were assayed essentially as described previously (7-9). The standard assay for DNA polymerase using poly(dA-dT) alternating copolymer as the template primer contained 0.025 A₂₆₀ O.D. units of activated poly(dA-dT), 40 mM TrisHCl (pH 7.5), 0.8 mM MgCl₂, 40 μ M dATP, 4 μ M [³H]dTTP (500 - 1000 cpm/pmole), 5 μ g bovine serum albumin, 2% glycerol, 0.04% Triton X-100, 2 mM dithiothreitol, and 0.2 - 0.5 units of DNA polymerase δ in a total volume of 125 μ l. The assay mixture was incubated at 37° C for 15 minutes and terminated by the addition of 5% trichloroacetic acid containing 10 mM sodium pyrophosphate. The precipitate was collected on

TABLE I. Effects of DMSO on Human Placental DNA Polymerases α and δ .

Addition	Polymerase δ	Polymerase α
None	100	100
Dimethylsulfoxide	560	33
N,N-dimethylformamide	280	37
Acetone	67	81
Ethanol	91	29
Triton X-100	106	109

DNA polymerase δ and α activities were measured by the incorporation of [³H]dTTP into activated calf thymus DNA, in the presence of the listed compounds at a final concentration of 15% v/v. Results are expressed as relative activities. Assays for DNA polymerase were performed as described in Experimental Procedures, using 0.05 units of DNA polymerase δ and 0.1 units of DNA polymerase α per assay in a 30 min incubation.

Whatman GF/C filters and counted. The standard assay when calf thymus DNA was used as the template primer contained 0.03 A₂₆₀ O.D. units of activated calf thymus DNA, 40 mM N-2-hydroxyethylpiperazine N'-2-ethanesulfonic acid, pH 7.5, 3 mM MgCl₂ 40 μM each of dATP, dGTP, and dCTP, 4 μM [³H]-dTTP (500 - 1000 cpm/pmole), 5 μg bovine serum albumin, 2% glycerol in a volume of 125 μl. One unit of activity was defined as the amount that incorporated 1 nmole deoxynucleotide into DNA per hour. Assays for 3' to 5' exonuclease activities were performed as previously described (7-9), by the release of [³H]dTTP from [³H]d(T)₅₀.

RESULTS

DNA polymerase δ activity was markedly increased in our standard assays when DMSO was present, whereas DNA polymerase α activities were reduced. (Table I). At a concentration of 15% v/v, polymerase δ activity was increased almost six-fold while polymerase α activity was reduced by two-thirds. N,N-dimethylformamide, another dipolar aprotic solvent, also increased polymerase δ activity, whereas Triton X-100, acetone or ethanol were ineffective (Table I). The concentration dependence of the effects of DMSO is shown in Fig. 1. When

Figure 1. Concentration Dependence of DMSO Effects on the Activity of DNA Polymerase δ.

DNA polymerase δ was assayed in the presence of the indicated concentrations of DMSO using poly(dA-dT) (●) or activated calf thymus DNA (▲) as the template primers. The effect of DMSO on the 3' to 5' exonuclease activity of polymerase δ was also determined (■). One hundred percent values were as follows: 50 pmoles (●) and 16 (▲) pmoles dTMP incorporated/hr in the polymerase assay, and 21 pmoles (■) dTMP released/hr in the exonuclease assay.

Figure 2. Concentration Dependence of the Effects of DMSO on the Activity of DNA Polymerase α and *E. coli* Pol I.

DNA polymerase α was assayed in the presence of the indicated concentrations of DMSO using poly(dA-dT) (■) or activated calf thymus DNA (▲) as the template primers, as shown in panel A. The effects of DMSO on *E. coli* Pol I assayed using poly(dA-dT) (■) or activated calf thymus DNA (▲) as the template primers are shown in panel B.

For panel A, 100 % values were 47 pmoles (■) and 13 pmoles (▲) dTMP incorporated per hr. For panel B, 100 % values were 738 pmoles (■) and 121 pmoles (▲) dTTP incorporated per hr.

poly(dA-dT) was used as the template primer, a maximal increase in activity of about four-fold was observed at about 20% DMSO. When activated calf thymus DNA was the template primer, the observed activation was less, about two-fold at 15% DMSO (Fig. 1). Since DNA polymerase δ has a 3' to 5' exonuclease activity, the effect of DMSO on this activity was also examined (Fig. 1). Only slight inhibition of the exonuclease activity was observed, precluding the possibility that the effects observed on polymerase activity are due to an inhibition of the exonuclease activity, since it is known that inhibition of the exonuclease activity leads to an increase in measurable dNMP incorporation (5-9). The concentration dependence of the inhibition of human placental DNA polymerase α by DMSO, assayed with either activated calf thymus or poly(dA-dT) as the template primer, is shown in Fig. 2A. The inhibition is nearly complete at 30% DMSO. In other experiments, we have observed that this inhibition cannot be accounted for by inactivation of polymerase α , since exposure to DMSO followed by dilution leads to restoration of the activity. A procaryotic DNA polymerase, *E. coli* Pol I, which also has an associated 3' to 5' exonuclease activity, was inhibited in a manner similar to that observed for polymerase α (Fig. 2B).

Figure 3. Lineweaver-Burk Plot of the DNA Polymerase Reaction in the Presence of DMSO.

DNA polymerase δ activity was assayed using poly(dA-dT) as the template primer at varying concentrations of dTTP, and at the following concentrations of DMSO : 0% ($\bullet$); 1% ($\blacktriangle$); 5% ($\blacksquare$); 10% ($\square$). Values of V are pmoles of dTTP incorporated per 30 min, and values of S are in μ M dTTP.

The apparent activation of placental DNA polymerase δ by DMSO at varying substrate (dTTP) concentrations was examined. The Lineweaver-Burk plots (Fig. 3) show that the apparent K_m for the substrate, dTTP, was significantly reduced while the V_{max} of the polymerase reaction was unaffected. In the presence of 20% DMSO, there was a five-fold reduction in the K_m for dTTP, from 3.5 μ M to 0.7 μ M (data not shown). Thus it appears that in the presence of DMSO, the apparent affinity of the enzyme for dTTP is increased. This accounts for the apparent activation of DNA polymerase δ which is observed, since the standard assay utilizes a nonsaturating concentration of labelled dTTP (4 μ M).

These findings also suggest that the addition of DMSO in the assay of DNA polymerase activity could provide a useful method for the selective assay of DNA polymerase δ . During the isolation of the latter, it is difficult to identify the polymerase δ enzyme fractions in the presence of polymerase α activities because of the presence of polymerase α activities; the 3' to 5' exonuclease activity, the main identifying characteristic of polymerase δ , cannot be reliably determined until after removal of the major portion of the nonassociated exonucleases (9). In preliminary work, we have observed that polymerase δ activity can be positively identified even during DEAE-cellulose chromatography of crude placental extracts by virtue of an increased activity in the presence of

Figure 4. Separation of a Mixture of Partially Purified DNA Polymerase α and δ on Heparin-Agarose.

A mixture of partially purified human placental DNA polymerase α and polymerase δ was chromatographed on a Heparin-Agarose column and eluted with a salt gradient. Fractions were assayed for DNA polymerase activity with no additions (●), in the presence of 20% DMSO (■), or in the presence of 10 μ M BuAdATP (▲).

20% DMSO, while polymerase α fractions are almost completely inhibited. The potential utility of this strategem is demonstrated by the experiment shown in Fig. 4, in which a mixture of partially purified DNA polymerase α and δ was chromatographed on heparin-agarose and the fractions assayed in the absence and presence of 20% DMSO. As can be seen in Fig. 4, DNA polymerase α , is extensively inhibited in the presence of DMSO, while polymerase δ activity is markedly increased. We have also recently shown that BuAdATP is a selective inhibitor of polymerase α (9); the effects of this inhibitor is also shown in Fig. 4 for comparison. It is seen that BuAdATP almost completely inhibits the polymerase α activity while the polymerase δ activity is only slightly affected.

DISCUSSION

The findings reported here are significant because they provide further evidence for a distinct identity of polymerase δ and α . We have recently reported that BuAdATP is a selective inhibitor of polymerase α , and suggested that it may be useful in distinguishing this enzyme activity from polymerase δ . Here we show that the use of DMSO in the assay may provide an additional means for assaying and identifying polymerase δ in the presence of polymerase α , with

a significant advantage that this is a readily available chemical compound.

The basis for the effects of DMSO on polymerase δ activity are not clear; DMSO affects both isolated enzymes and intact cell systems by a number of diverse mechanisms (see ref. 12,13 and others therein). The fact that this property is shared with dimethylformamide may be related to the fact that both are dipolar aprotic solvents; DMSO may replace water in the hydration sphere of proteins and thus induce conformational changes which affect their biological activities (12). A number of other enzymes have been described whose activity is affected by DMSO, including RNA polymerase (14) and tyrosine protein kinase (15). This present report, however, is the first to describe the effect of this agent on a mammalian DNA polymerase. Moreover, many cellular effects of DMSO have been described which involve either cell proliferation and/or differentiation (16,17), so that DNA polymerase activities may be a potential locus of its actions.

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