THE TEMPLATE SPECIFICITIES OF DNA POLYMERASE IN CELL FREE LYSATES OF XENOPUS LAEVIS EGGS, LARVAE AND IMMATURE OVARIES

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Received June 28, 1973

SUMMARY

DNA polymerase activities in cell-free lysates of unfertilized eggs, larvae and immature ovaries of Xenopus laevis were compared to purified E. coli DNA polymerase I using several natural and synthetic templates. The templates were tested as the native and denatured forms of normal and DNase I treated molecules. Although the Xenopus polymerases tended to prefer DNase I treated Xenopus DNA over the other templates tested, so did the E. coli polymerase I. In general, the template preferences of the polymerases studied depended in complex ways on both the form and the species of origin of the template.

INTRODUCTION

The substrate specificities of polynucleotide metabolic enzymes are determined by comparing the initial rates of enzyme action on different substrates under conditions such that the reaction is enzyme limited. Particular enzymes select substrates on the basis of diverse properties; for example, one might react with single-stranded DNA only, another with RNA only (1). Higher orders of specificity are shown by the restriction endonucleases, which recognize particular short nucleotide sequences (2) or selectively degrade DNA from any source other than the bacterium that makes the endonuclease (3). Similar higher order specficity is conceivable for DNA polymerases, but evidence for its existence is weak. Although studies of the template preferences of DNA polymerases from many sources have been done (4-19), only one polymerase, from rat liver mitochondria, has shown a preference for DNA from the same source (4), and that result was not confirmed (5).

The activity of a DNA polymerase is known to depend on the physical form

of the template, the number and nature of initiation sites, divalent cation concentration, ionic strength, concentration of precursors, and the reaction temperature. All of these factors must be controlled to make meaningful comparisons of polymerase activity on DNA from different sources. Thus we hypothesized that if the templates were prepared in ways that made their physical form as uniform as possible and if the other variables mentioned were held constant, differences in the rate of nucleotide incorporation by the polymerases studied would be due to differences in the composition of the template traceable to the source of the template. Specifically, one or more of the Xenopus polymerases might prefer Xenopus DNA much more strongly than would the E. coli polymerase.

We chose to study the Xenopus laevis (African clawed toad) polymerases after Gurdon, et al., (17) reported the existence of a DNA polymerase in unfertilized eggs of Xenopus and showed evidence that this enzyme(s) had preferences for templates that depended on their form and species of origin. Pestell extended this work to lysates of unfertilized eggs and larvae of Xenopus (18). Xenopus polymerases were compared with highly purified E. coli polymerase I [Kornberg Fraction VII (19)]. We chose to work with centrifuged cell lysates rather than highly purified enzymes in the expectation that species specificity was likely to require factors in addition to the polymerase molecule. To check this assumption, we purified one of the polymerases 50-fold.

EXPERIMENTAL PROCEDURES

Crude extracts of Xenopus eggs and larvae were obtained as described by Pestell (18), except that all buffers included 10% glycerol and the homogenizing buffer contained 0.25% Triton X-100. Ovaries were removed from female toads (20) 17 to 23 days after metamorphosis so that a majority of the oocytes were in the pachytene stage of development and were selectively replicating their ribosomal DNA (21). After removal, the ovaries were washed, homogenized in a Virtis homogenizer in 20 mM Tris-Cl pH 7.5, 1 mM EDTA, 5 mM 2-mercapto-

ethanol, 10% glycerol, 0.25% Triton X-100, and thereafter treated the same as the egg and larval extracts. The egg polymerase was purified by phosphocellulose batch filtration to remove contaminating proteins followed by DEAE-cellulose batch filtration to remove nucleic acids. The polymerase activity eluted from a phosphocellulose column between 0.30 and 0.42M phosphate (pH 7.5, 1 mM EDTA, 5 mM 2-mercaptoethanol, 10% glycerol). Enzymes were stored at -196°C. Calf thymus DNA (type I) was purchased from Sigma; M. lysodeikticus DNA from Miles Laboratories; E. coli and Cl. perfringens DNA from Worthington; and d(A-T) copolymer from Biopolymers. These DNAs were used without further purification. B. subtilis and Xenopus erythrocyte DNAs were extracted and purified according to the method of Marmur (22), except that each RNase treatment was followed by treatment with pronase (grade B, Calbiochem). The DNA from each source was treated for various lengths of time at 22°C with pancreatic endonuclease I (Worthington). The nuclease was inactivated by addition of citrate and heating, and the activity of purified egg polymerase on the treated DNA was assyed. The activity increased for a short time as the number of initiation sites increased and then fell off slowly as the molecular weight decreased. We chose the maximally active aliquot of DNA from each source as the "activated" DNA from that source. Both untreated and "activated" DNAs were denatured by heating to 5°C above their melting temperature (23). E. coli polymerase I (gift of Dr. D. Brutlag) was assayed as described by Richardson, et al., (19). The reaction mixture for the Xenopus polymerase (0.25ml) contained 12 nmole DNA, 7.5 μmole NaCl, 0.75 μmole trisodium citrate, 3.0 nmoles each of dATP, dCTP, and dGTP, 1.5 nmole ^{3}H-dTTP (0.67 Ci/mmole from New England Nuclear), 2.5 μmole $MgSO_4$, 2.0 μmole phosphate buffer (pH 7.5), 1.0 μmole 2-mercapthoethanol, 0.10 μmole EDTA and $\leq$0.03 units of enzyme. Assays were incubated 20 minutes at 37°C, stopped and filtered as by Pestell (18). One unit of enzyme catalyzes the incorporation of 10 nmoles of total nucleotide into acid precipitable form in 30 minutes. Control assays omitting dATP, dCTP, and dGTP; template; enzyme; or Mg^{++} gave backgrounds of <2% of the maximum activity.

RESULTS

The purification of the DNA polymerase activity from unfertilized eggs of Xenopus is summarized in Table I. The enzyme lost negligible activity after

TABLE I

Purification of DNA Polymerase from
Unfertilized Eggs of Xenopus laevis

	Fraction	Volume (ml)	Protein (mg/ml)	Specific Activity (units/mg)	Total Units
I.	Crude Extract	50.0	11.0	0.0086	4.8
II.	Phosphocellulose Batch Filtration.	142.0	2.2	0.033	10.4
III.	DEAE - cellulose Batch Filtration.	240.0	0.64	0.218	33.4
IV.	Phosphocellulose Chromography	5.0	2.1	0.463	4.5

one week at 4°C in Tris or phosphate buffers containing mercaptoethanol and 10% glycerol, and lasted indefinitely at temperatures below -20° C. Fraction IV showed very low activity when Tris buffer was substituted for phosphate in the assay cocktail. This effect was not observed with any other polymerase fraction tested.

The relative activities of the several enzymes on selected templates are shown in Figure I. The template preferences of the enzymes were the same for different preparations of enzymes and templates. The variation of the scale

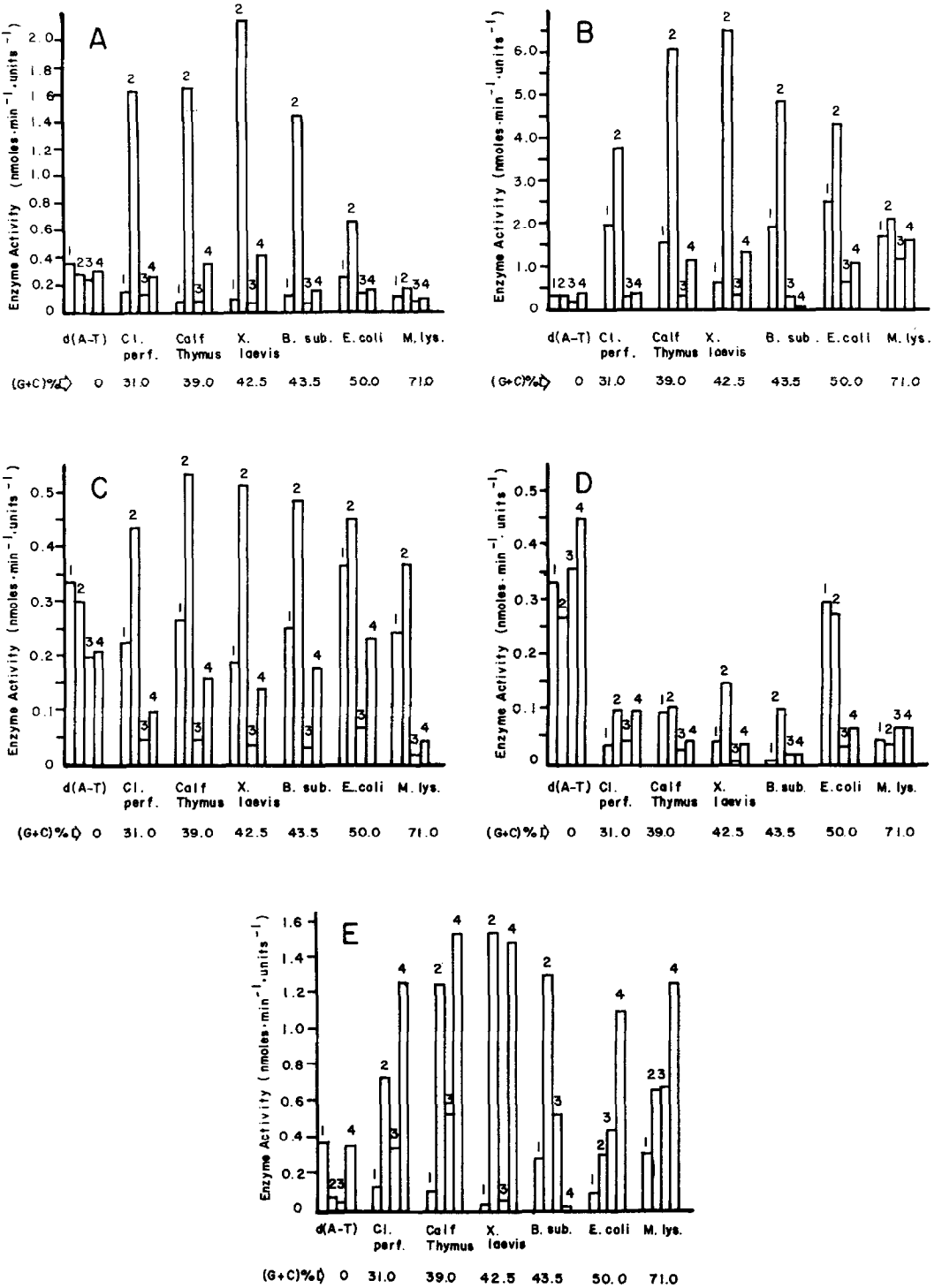
A
B
C
D
E
Enzyme Activity (nmoles·min⁻¹·units⁻¹)
d(A-T)
Cl. perf.
Calf Thymus
X. laevis
B. sub.
E.coli
M. lys.
(G+C)%
0
31.0
39.0
42.5
43.5
50.0
71.0

Fig. 1.

of ordinates for the various enzymes reflects the variation of the activities of the enzymes with d(A-T) copolymer as a template, since the number of units of enzyme per assay were computed with d(A-T) template. An estimate of the total error inherent in the assay can be obtained by comparing the results for bihelical and denatured d(A-T) because d(A-T) renatures as soon as it is cooled.

DISCUSSION

We have extracted DNA polymerases from Xenopus laevis at different developmental stages and measured their abilities to utilize DNA templates in several forms from several sources. We found that the egg (both crude and 50-fold purified) and larval polymerases strongly prefer bihelical to denatured DNA, particularly as compared to E. coli polymerase I. The polymerase(s) from pachytene ovaries is quite distinct showing no strong template form preference but singular preferences for d(A-T) and E. coli DNA.

The Xenopus polymerases tested do not show the kind of higher order substrate specificity of the restriction endonucleases. We cannot, however, rule out all possibility that under the right conditions one of the polymerases tested would show specificity for its natural substrate. The reasons for this inability are: first, we tested only one of the four classes of ribose and deoxyribose template-initiator complexes described by Chang and Bollum (6); second, we used three of the four active forms in which such a template-initiator complex can be formed (24); third, purification of the Xenopus DNA may have removed factors necessary for specific DNA-polymerase reaction; and fourth, the relative activities of a given Xenopus polymerase on templates from various sources might be dependent on the ionic strength or divalent cations used in the assay cocktail as has been

Caption for Figure I.

Specific activities of Xenopus and E. coli DNA polymerase with selected templates. The form of template is indicated by the numerals above the bars as follows: 1) untreated, 2) DNase I treated, 3) denatured, and 4) DNase I treated, denatured; A) crude extract of Xenopus eggs. B) 50-fold purified lysate of Xenopus eggs. C) lysate of Xenopus larvae. D) lysate of Xenopus immature ovaries. E) E. coli DNA polymerase I.

shown for mitochondria from rat liver (5) and tetrahymena (16).

Finally, most characterizations of DNA polymerases include statements about their template preferences. We have found that such statements must be considered very carefully because the preference of a DNA polymerase for template from a certain source over template from another source is dependent on how the templates are prepared, and conversely, preference for template of a certain form (bihelical vs. denatured, for example), may be dependent on the source of the template.

This work was supported by grants from N.I.H. (AI06160 and GM00295) and the National Cystic Fibrosis Foundation. This topic was suggested by Prof. J. Lederberg, for whose support we are grateful. We also thank Prof. A. T. Ganesan for his help.

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