Volume 280, number 2, 281–283 FEBS 09512 March 1991

ADONIS 001457939100219Y

Eukaryotic DNA primase

Abortive synthesis of oligoadenylates

V.N. Podust, O.V. Vladimirova, E.N. Manakova and O.I. Lavrik

Institute of Bioorganic Chemistry, Siberian Division of the USSR Academy of Sciences, Novosibirsk 630090, USSR

Received 11 January 1991

Calf thymus DNA polymerase α-primase, human placenta DNA polymerase α-primase and human placenta DNA primase synthesized oligoriboadenylates of a preferred length of 2–10 nucleotides and multimeric oligoribonucleotides of a modal length of about 10 monomers on a poly(dT) template. The dimer and trimer were the prevalent products of the polymerization reaction. However, only the oligonucleotides from heptamers to decamers were elongated efficiently by DNA polymerase α.

DNA primase; DNA polymerase α; Primer length

1. INTRODUCTION

DNA replication in vivo requires preliminary formation of oligoribonucleotides of about 10 monomers in length, which serve as primers for the synthesis of Okazaki fragments [1–3]. DNA primase, an enzyme responsible for the synthesis of RNA primers was purified from various sources both as separate enzyme and in complex with DNA polymerase α [4–9], (for earlier references see [10]). This enzyme was shown in vitro to synthesize processively discrete-length oligoribonucleotides of an apparent length of 7–12 monomers and multimeric oligoribonucleotides of a model length of about 10 monomers [4–7,11–21]. We show here, that in the presence of ATP substrate and poly(dT) template, DNA primases synthesize oligoriboadenylates from dimer to decamer and multimeric oligoribonucleotides, the dimer and trimer being the prevalent products. However, only the oligonucleotides from heptamers to decamers were elongated efficiently by DNA polymerase α.

Correspondence address: V.N. Podust, Institute of Bioorganic Chemistry, Siberian Division of the USSR Academy of Sciences, Novosibirsk 630090, USSR

Abbreviations: PAGE, polyacrylamide gel electrophoresis; CT (HP) polα-primase, calf thymus (human placenta) DNA polymerase α-primase; HP primase; human placenta DNA primase; XC, xylenecyanol; BB, Bromophenol blue

2. MATERIALS AND METHODS

Poly(dT) was from Sigma, other chemicals were of analytical grade. [α-^{32}P]ATP was from Radiopreparat (USSR). Before use the nucleotide was purified by PAGE as described below. Monoclonal antibody SJK 287-38 was a kind gift of F. Grosse (Göttingen, Germany).

CT polα-primase and HP polα-primase were purified to near homogeneity as described [9,22]. HP primase was purified as HP polα-primase complex until binding to immunoaffinity column. Primase was eluted with 50 mM Tris-HCl, pH 8.6, 1 M KCl, 1 mM 2-mercaptoethanol and dialyzed vs 20 mM Tris-HCl, pH 7.5, 4 mM 2-mercaptoethanol, 50% v/v) glycerol and stored at −20°C. HP primase preparations contained only 52 kDa and 59 kDa polypeptides as shown by SDS gel electrophoresis. One unit of primase activity was defined was in [9].

Reaction mixtures for primase activity contained 20 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 1 mM dithiothreitol, 0.5 A_{267}/ml poly(dT), 0.5 mM [α-^{32}P]ATP (1–3 Ci/mmol). 30 μl of the assay mixture was incubated with 1–3 U of primase at 37°C for 1 h followed by precipitation of the nucleotide material with 0.3 ml of cold 2% $NaClO_4$ solution in acetone. After centrifugation, the pellets were washed twice with cold acetone, dried and redissolved in 7 M urea containing tracking dyes XC and BB. The samples were electrophoresed on 20% polyacrylamide gels in 7 M urea, 0.05 M Tris-borate, pH 8.3, 1 mM EDTA followed by autoradiography. Oligonucleotide bands in gel slabs were quantitated either by densitometrical scanning of autoradiograms ('Ultroscan XL', LKB), or by scintillation counting of the excised gel strips. Both methods gave similar results. Optical absorbance of spots of the autoradiographs prepared for scanning was below 2.

Autoradiographically localized nucleotide material was extracted, if necessary, from the gel onto DE-81 paper (Whatman), eluted from the paper with 1 M $NaClO_4$ and precipitated with acetone.

3. RESULTS AND DISCUSSION

A commonly accepted model of chromosomal DNA replication suggests a mechanism in which DNA primase synthesizes an RNA primer de novo that serves

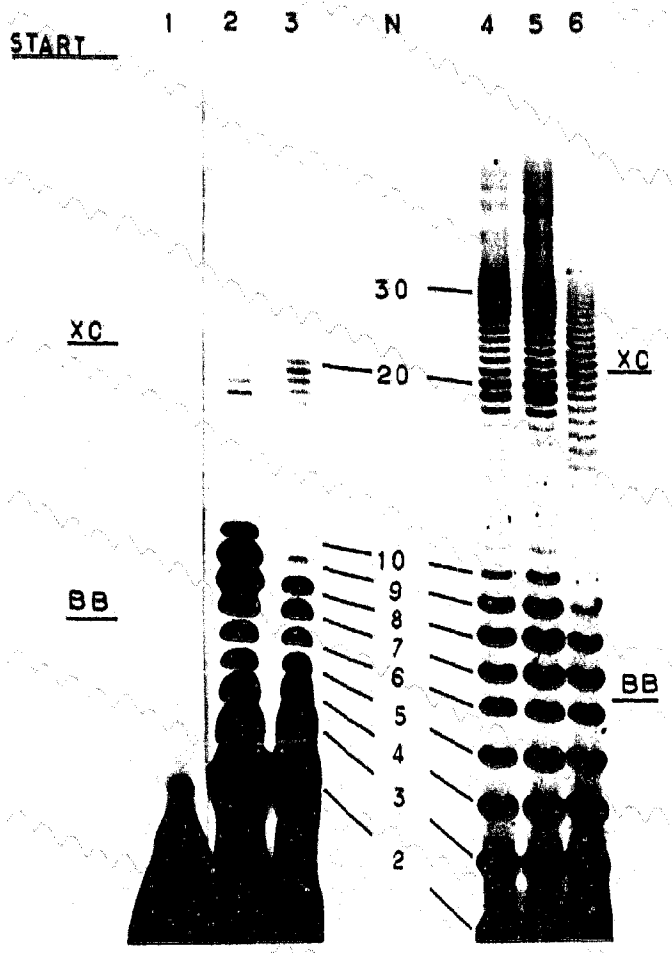

Fig. 1. PAGE analysis of oligoriboadenylates synthesized by CT polα-primase (lanes 2 and 3), HP primase (lane 4), HP polα-primase (lanes 5 and 6). The reaction mixture was incubated without enzyme (lane 1), without dATP (lanes 2, 4 and 5) or in the presence of 50 μM dATP (lanes 3 and 6). The positions of the tracking dyes BB and XC are indicated.

as a substrate for DNA polymerase. The length of primers synthesized was an object of numerous investigations [4-7,11-21]. It was evaluated either using DNase degradation of products synthesized by DNA-polymerase-primase or by direct measurement of RNA products polymerased by primase. The enzyme was shown to synthesize oligoribonucleotides of an apparent length of 7–12 monomers and multimeric oligoribonucleotides of a modal length of about 10 monomers. These results were interpreted in favour of a processive manner of polymerization. Processivity of nucleic acid synthesis assumes recurrent polymerization steps before dissociation of a polymerase from a complex with stable template-primer duplex. However, processive growth of a polynucleotide chain may be disturbed during initiation or short primer elongation. Processive enzymes, such as human placenta DNA polymerase α [23], avian myeloblastosis virus revertase [24] and *E. coli* RNA polymerase [25,26] synthesize dimers and trimers from mononucleotides. Supposingly, di- and trinucleotides do not form long-living complexes and easily dissociate from the enzyme before the next polymerization step. Since primases construct oligonucleotides from monomers, they are bound to overcome the critical formation of di- and trimers.

We have analysed for the first time the oligoriboadenylates synthesized by HP polα-primase and HP primase on poly(dT) template. The properties of human and bovine primases were compared. The data obtained are in good agreement with earlier observations (Fig. 1). All primase preparations used synthesized oligonucleotides up to 10-mer (monomeric oligomers) and multiples of a modal length of 10 nucleotides (multimeric oligomers). However one essential observation was made. Primase, be it in complex with DNA polymerase α or in free state, produced a whole spectrum of oligoadenylates from dimer to decamer. Moreover, dimer and trimer are the predominant products of the reaction (Figs. 1 and 2). Control experiments were carried out (not shown). No oligomerization of ATP was observed in the absence of primase. Product composition did not depend on template concentration (we raised the poly(dT) concentration to 5 A_{267}/ml). All primase preparations were essentially free from nuclease activity. We eluted 8–9-mers from the gel and reincubated them with the primase reaction mixture as described in section 2 (ATP was omitted) for 1.5–4 h. PAGE analysis showed no noticeable cleavage of oligonucleotides.

Two experimental conditions are principal here: (1) commercial [α-^{32}P]ATP preparations require additional purification by PAGE, otherwise the observable dimerpentamer bands in autoradiography are masked by impurities; (2) all treatments of samples before PAGE must exclude any possibility to lose nucleotide material. We precipitated the nucleotides using a cold 2% $NaClO_4$ solution in acetone. This procedure recovers nearly all radioactivity including [α-^{32}P]ATP.

The addition of 50 mM dATP to the reaction mixtures led to inhibition of multimeric oligonucleotide synthesis and a decrease in relative content of 7–10-mers and accumulation of the polymerized products (Fig. 1, lanes 3 and 6). This result confirms the model in which DNA polymerase α elongates the primers of average length about 10 nucleotides.

We show here that the predominant products of primase polymerization reaction are dimer and trimer. The processive synthesis of the 'mature' primers occurs, if at all, with rather low efficiency. A distributive stepwise elongation of short oligoriboadenylates can not be ruled out. A considerable amount of primase product is abortive, not further utilized by DNA polymerase α. We suppose that the formation of such short products may be a common feature of template-dependent polymerases.

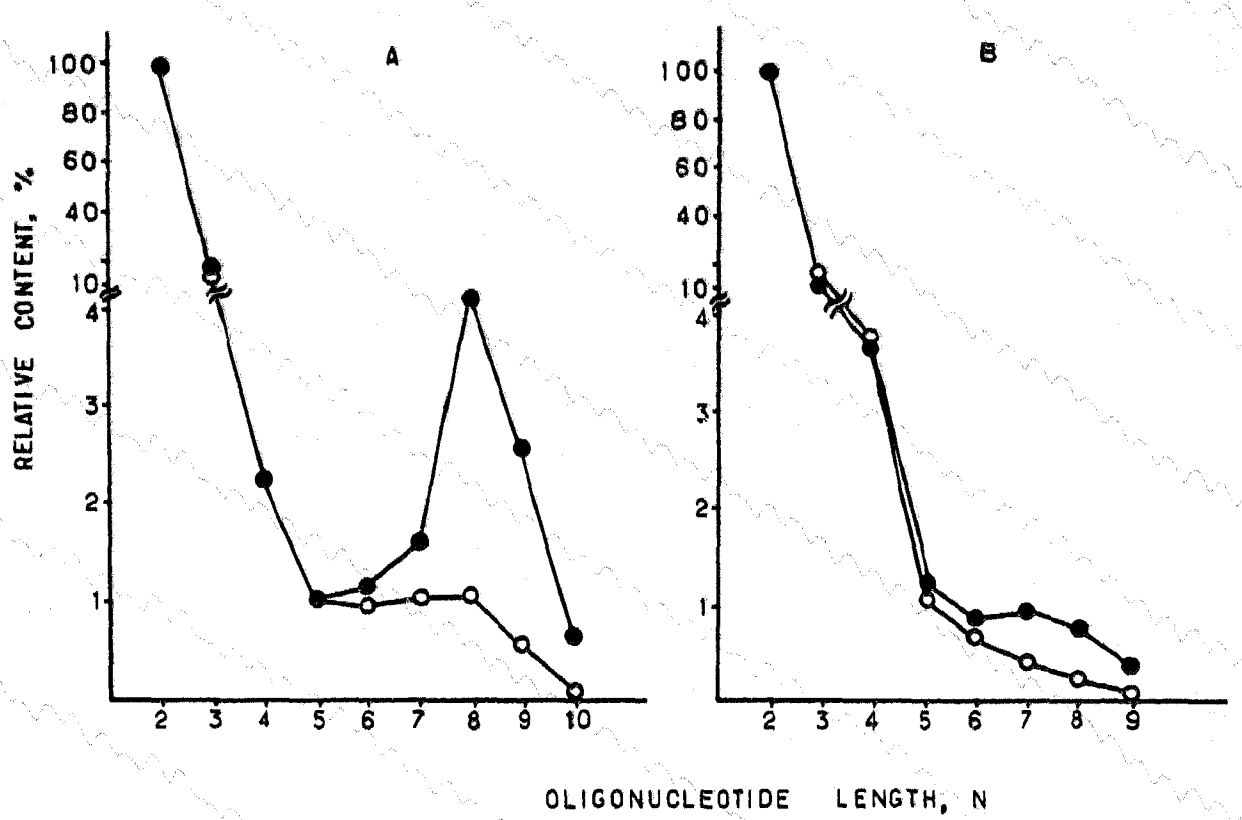

Fig. 2. The dependence of relative content of oligoriboadenylates synthesized by CT polα-primase (A) and HP polα-primase (B) on the oligonucleotide length. The reaction mixture was incubated without dATP (●) or in the presence of 50 μM dATP (○). Measured values of oligonucleotide content were reduced to the number of phosphate groups per molecule. The values for dimer were considered as 100%.

REFERENCES

[1] Kornberg, A. (1980) DNA Replication, Freeman, San Francisco.
[2] DePamphilis, M.L. and Wassarman, P.M. (1980) Annu. Rev. Biochem. 49, 627–666.
[3] Kitani, T., Yoda, K.Y. and Okazaki, T. (1984) Mol. Cell. Biol. 4, 1591–1596.
[4] Biswas, E.E., Joseph, P.E. and Biswas, S.B. (1987) Biochemistry 26, 5377–5382.
[5] Nasheuer, H.-P. and Grosse, F. (1988) J. Biol. Chem. 263, 8981–8988.
[6] Bialek, G., Nasheuer, H.-P., Goetz, H., Behnke, B. and Grosse, F. (1988) Biochim. Biophys. Acta 951, 290–297.
[7] Brooks, M. and Dumas, L.B. (1989) J. Biol. Chem. 264, 3602–3610.
[8] Goulian, M. and Heard, C.J. (1989) J. Biol. Chem. 264, 19407–19415.
[9] Podust, V.N., Lavrik, O.I., Nasheuer, H.-P. and Grosse, F. (1989) FEBS Lett. 245, 14–16.
[10] Roth, Y.-F. (1987) Eur. J. Biochem. 165, 473–481.
[11] Tseng, B.Y. and Ahlem, C.N. (1982) J. Biol. Chem. 257, 7280–7283.
[12] Tseng, B.Y. and Ahlem, C.N. (1983) J. Biol. Chem. 258, 9845–9849.
[13] Hu, S.-Z., Wang, T.S.-F. and Korn, D. (1984) J. Biol. Chem. 259, 2602–2609.
[13a] Gronostajski, R.M., Field, J. and Hurwitz, J. (1984) J. Biol. Chem. 259, 9479–9486.
[14] Yamaguchi, M., Hendrickson, E.A. and DePamphilis, M.L. (1985) J. Biol. Chem. 260, 6254–6263.
[15] Wilson, F.E. and Sugino, A. (1985) J. Biol. Chem. 260, 8173–8181.
[16] Philippe, M., Rossignol, J.-M. and De Recondo, A.-M. (1986) Biochemistry 25, 1611–1615.
[17] Vishwanatha, J.K. and Baril, E.F. (1986) Nucleic Acids Res. 14, 8467–8487.
[18] Singh, H., Brooke, G., Pausch, M.H., Williams, G.T., Trailor, C. and Dumas, L.B. (1986) J. Biol. Chem. 261, 8564–8569.
[19] Badaracco, G., Valsasnini, P., Foiani, M., Benfante, R., Luccini, G. and Plevani, P. (1986) Eur. J. Biochem. 161, 435–440.
[20] Cotterill, S., Chui, G. and Lehman, I.R. (1987) J. Biol. Chem. 262, 16105–16108.
[21] Suzuki, M., Enomoto, T., Masutani, C., Hanaoka, F., Yamada, M. and Ui, M. (1989) J. Biol. Chem. 264, 10065–10071.
[22] Nasheuer, H.-P. and Grosse, F. (1987) Biochemistry 26, 8458–8466.
[23] Nevinsky, G.A., Veniaminova, A.G., Levina, A.S., Podust, V.N., Lavrik, O.I. and Holler, E. (1990) Biochemistry 29, 1200–1207.
[24] Lokhova, I.A., Nevinsky, G.A., Bulychev, N.A., Gorn, V.V., Levina, A.S., Rudenko, N.K., Kavsan, V.M. and Lavrik, O.I. (1990) Mol. Biol. (USSR) 24, 396–407.
[25] Carpousis, A.J. and Gralla, J.D. (1980) Biochemistry 19, 3245–3253.
[26] Grachev, M.A. and Zaychikov, E.F. (1980) FEBS Lett. 115, 23–26.