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DNA polymerase activity in water-structured and confined environment of reverse micelles

Rashid O. Anarbaev *, Svetlana N. Khodyreva, Alexandra L. Zakharenko, Nadejda I. Rechkunova, Olga I. Lavrik

Institute of Chemical Biology and Fundamental Medicine, Siberian Division of Russian Academy of Sciences, Prospect Lavrentiev 8, 630090 Novosibirsk, Russian Federation

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Abstract

DNA polymerases are the key enzymes of DNA replication and repair. These proteins in living cells are functioning not as isolated entities but in multiprotein complexes, and their work are carefully regulated. The main factors determining the enzyme activity are the structure and dynamics of 'biological' water. Reverse micelles (nano-sized water droplets dispersed in a continuous oil phase) are the simple model systems where the structure and dynamics of water are controlled. In this work, the activities and processivity of Klenow fragment of *E. coli* DNA polymerase I, thermostable *Tte* DNA polymerase, and HIV-1 reverse transcriptase are investigated as a function of the water pool size. Klenow fragment was more active on poly(rA)-oligo(dT) in reverse micelles compared with the water buffer with activity being increased upon increasing water content. *Tte* polymerase was more active at low water content. HIV-1 reverse transcriptase revealed comparable activity and processivity on poly(rA)-oligo(dT) in the water buffer and reverse micelles at water content of 15–40%. Thus, the polymerase activity appears in certain range of water concentration and depends on the local polarity determining the protein 'expansion', microviscosity inside nano-droplets determining the enzyme dynamics, and nucleic acid hydration degree.

Keywords: DNA polymerase; Enzymatic nucleic acids synthesis; Reverse micelles

1. Introduction

Biochemical assays are typically performed using very dilute solutions of macromolecular components. On the other hand, most of the enzymes in the living system are working in an environment, which is quite different from aqueous bulk solution. Water is an essential component in protein structure, function, and dynamics [1–3]. Biomembrane and bound water existing near the boundary surface must affect the catalytic activity of enzymes in cells more or less, resulting in regulation of the enzyme function. An experimental approach that allows the study of water–protein interactions with different, but low content of water is to entrap proteins in the interior of reverse micelles [4–7]. Reverse micelles, or water-in-oil microemulsions, are nano-meter-sized water droplets dispersed in an apolar solvent with the aid of a surfactant monolayer, forming a thermodynamically stable and optically transparent solution. The main feature of these systems is their ability to solubilize both hydrophilic and hydrophobic substances, which are localized within their different distinct microdomains. In these systems it is possible to vary the size of the micelles and, hence, the amount of water in contact with the protein. Reverse micelles have been used as a model system for biological studies ranging from basic biochemical research [4,8] to applied biotechnology [9,10].

Abbreviations: *Tte* pol, DNA polymerase from *Thermus thermophilus* B35; *Tth* pol, DNA polymerase from *Thermus thermophilus* HB8; *Taq* pol, DNA polymerase from *Thermus aquaticus* YT1; CTAB, cetyl trimethylammonium bromide; Brij58, polyoxyethylene 20 cetyl ether; Brij30, polyoxyethylene 4 lauryl ether; SDS, sodium dodecylsulfate; Triton X-100, polyoxyethylene 9.5 *p-tert*-octylphenyl ether

* Corresponding author. Tel.: +7 3832309296; fax: +7 3832333677.
E-mail address: anarbaev@niboch.nsc.ru (R.O. Anarbaev).

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Earlier, we have found that DNA polymerase α-primase complex and Klenow fragment of *E. coli* DNA polymerase I synthesize DNA on synthetic templates in reverse micelles. The enzyme activity was observed in micelles stabilized by non-ionic surfactants and their mixtures with other types of detergents. But DNA polymerases revealed a maximal activity in the system composed of cationic CTAB, anionic SDS, non-ionic Triton-X-114 and Brij 58 surfactants (concentration of 15, 25, 128, and 10 mM, respectively) in hexanol–decane (1:12 v/v). An increase in water content resulted in an increase of DNA polymerase processivity. The enzymes, however, synthesized more products in the water buffer than in reverse micelles [11].

In this work we significantly improved the above system by modifying composition of microemulsion and reaction conditions. In addition, we have investigated three different enzymes, namely Klenow fragment of *E. coli* DNA polymerase I, thermostable *Tte* DNA polymerase, and HIV-1 reverse transcriptase. Despite of different properties and peculiarities in DNA synthesis these enzymes have a similar three-dimensional structure, which assumes a hand-like structure [12–14]. The fingers, palm and thumb subdomains form the template-binding cleft and may influence the processivity of DNA synthesis [15–17]. Since the structure of the nucleic acid substrate depends on water content, we also studied the similarities and differences of catalytic activities of DNA polymerases on RNA and DNA templates. All the enzymes displayed high activity and processivity on these templates in reverse micelles at defined water content.

2. Materials and methods

2.1. Materials

Ammonium persulfate, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), acrylamide, *N*,*N*′-methylene-bis-acrylamide, poly(dT), poly(dA), poly(rA), dTTP, dATP, CTAB, Brij 30, Brij 58, Triton X-100 were purchased from Sigma (USA). $MgCl_2$ was from Merck (USA). Tris, bromophenol blue, dithiothreitol were obtained from Serva (Germany). [γ-^{32}P]ATP (3000 Ci/mmol) was purchased from Biosan (Russia). Klenow fragment of *E. coli* DNA polymerase I was from SibEnzyme (Russia). The oligonucleotides $(rA)_{15}$ and $(dT)_{16}$ were synthesized and kindly supplied by Dr. Abramova T.V. (Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences). Other chemicals were of chemical-grade purity or extra pure products.

Recombinant HIV-1 reverse transcriptase heterodimer (p66/p51) was isolated from *E. coli* DH5 strain harboring the plasmid pUC12N [18]. Recombinant *Tte* pol was purified from *E. coli* BL21 strain carrying plasmid pGT12 [19].

2.2. Separation of oligonucleotides by denaturing polyacrylamide gel electrophoresis

The nucleotide material was precipitated with 0.3 ml of 2% $LiClO_4$ in acetone at 0 °C. The precipitate was centrifuged at $10^4 \times g$ for 10 min, washed with cold acetone (0.3 ml), dried and dissolved in 5 μl of 7 M urea (in water) containing 0.5% bromophenol blue as a marker dye.

Electrophoresis was performed on vertical plates (15 cm × 25 cm × 0.03 cm). The polymerization mixture contained 50 mM Tris–borate buffer, pH 8.3, 20% acrylamide, 1% *N*,*N*′-methylene-bis-acrylamide, 7 M urea, 0.1% TEMED, and 0.05% ammonium persulfate. Electrophoresis was continued at 10 W until the marker dye migrated to the 3/4 of distance. The positions of radioactive oligonucleotides were located by autoradiography.

2.3. Preparation of reverse micelles

The microemulsion solutions were prepared by adding the measured volumes of hexanol and octane (1:6 v/v) to dried, pre-weighted amounts of Brij30, Triton X-100, SDS, CTAB, Brij 58 (concentration of 133, 77, 22, 11 and 7 mM, respectively), and further injecting the required volume of the water buffer. The mixture was shaken until being optically clear (1–5 s).

2.4. DNA polymerase and HIV-1 reverse transcriptase assays in the water buffer

Reaction mixtures (0.1 ml) contained 50 mM Tris–HCl, pH 8.0, 10 mM $MgCl_2$, 1 mM dithiothreitol, 25 μM dTTP, 10 μM (in all the cases the concentration of polynucleotide is as mononucleotide concentration) poly(dA) or poly(rA), 0.1 μM 5′-[^{32}P]$(dT)_{16}$ (specific activity of 1 Ci/μmol), and 5–10 nM DNA polymerase. Reactions were initiated by adding the enzyme. The assays were performed at 22 °C for 20 min and quenched with 10 volumes of 2% $LiClO_4$ in acetone. The samples were treated, and electrophoresis was performed as described above. Polymerization products were detected by autoradiography.

2.5. Klenow fragment assays in the water buffer on poly(dT) template

Reaction mixtures (0.1 ml) contained 50 mM Tris–HCl, pH 8.0, 10 mM $MgCl_2$, 1 mM dithiothreitol, 25 μM dATP, 10 μM poly(dT), 0.1 μM 5′-[^{32}P]$(rA)_{2–15}$ (specific activity of 1 Ci/μmol), and 5–10 nM DNA polymerase. Reactions were initiated by adding the enzyme. The assays were performed at 22 °C for 20 min and quenched with 10 volumes of 2% $LiClO_4$ in acetone. The samples were treated, and electrophoresis was performed as described above. Polymerization products were detected by autoradiography.