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DNA polymerase β reveals enhanced stability in reverse microemulsions

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ABSTRACT

Water is essential for the stability and functions of proteins and DNA. Reverse microemulsions are model systems where the structure and dynamics of water are controlled. We have investigated the different hydration and confinement effects on the activity and the stability of mammalian DNA polymerase β in the complex reverse microemulsions, containing ionic and nonionic surfactants in decane/hexanol. The enzyme displays high processivity on primed single-stranded M13mp19 DNA with maximal activity at 10% of water content. DNA polymerase reveals the enhanced stability toward the thermal and the chemical denaturation. The enzyme is still active at 65 °C and in 4 M urea. The data provide direct evidence for strong influence of microenvironment on DNA polymerase activity and stability.

1. Introduction

Water is essential for the stability and functions of proteins and DNA [1–3]. Hydration plays a major role in the assembly of a protein structure and dynamics. In living systems, the interactions between water and biopolymer occur in restricted geometries in cells and organelles. Generally, the properties of purified macromolecules have been studied in dilute solutions. On the other hand, biological macromolecules have evolved over billions of years to function inside cells, so researchers studying the properties of such molecules in vitro systems, ignore factors that reflect the intracellular environment. There are several universal aspects of the cellular interior that is largely neglected—the macromolecules crowding [4–6], confinement, and the altered structure and properties of water.

Reverse microemulsions (hydrated reverse micelles) allow the study of water–protein interactions with different water content [7–10]. Reverse microemulsions are transparent, isotropic, and thermodynamically stable liquid media with nanosized water droplets dispersed in a continuous oil phase and stabilized by surfactant molecules at the water–oil interface. They are the model systems where the structure and dynamics of water and confinement are controlled by changing the molar ratio of water to surfactant.

DNA polymerase β is one of the smallest nuclear eukaryotic DNA polymerases (molecular mass of 39 kDa) with a host of biochemical properties that make this enzyme an ideal model for studying the detailed mechanism of enzymatic DNA polymerization. DNA polymerase β has been suggested to play a role in DNA repair, DNA replication, and recombination [11–13].

In our previous papers [14,15], we have investigated the effect of different compositions of reverse microemulsions on activity of DNA polymerases and have found the system optimal for polymerase activity. In the present work, we investigate the influence of confinement on DNA polymerase β activity and stability.

2. Experimental

2.1. Materials

Ammonium persulfate, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), acrylamide, *N*,*N*′-methylene-bis-acrylamide, bromophenol blue, dithiothreitol, Tris, dNTP, cetyl trimethylammonium bromide (CTAB), polyoxyethylene 4 lauryl ether (Brij30), polyoxyethylene 20 cetyl ether (Brij58), polyoxyethylene 9.5 *p*-*tert*-octylphenyl ether (Triton X-100), sodium dodecylsulfate (SDS), fluorescein were purchased from Sigma. $MgCl_2$ was from Merck. [γ-^{32}P]ATP (3000 Ci/mmol) was purchased from Biosan (Russian Federation). The oligonucleotide 5′-GGCGATTAAGTTGGG (primer), single-stranded M13mp19 DNA, and T4 polynucleotide kinase was purchased from SibEnzyme (Russian Federation). The primer was labeled with [γ-^{32}P]ATP using polynucleotide kinase according to

Abbreviations: 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.

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the standard protocol [16] and annealed to M13mp19 DNA at position 6338–6352. Recombinant DNA polymerase β was purified from *E. coli* BL21DE3 pLys S harboring the plasmid pRSET [17].

2.2. Preparation of reverse microemulsions

The reversed microemulsions were prepared by adding the measured volumes of decane and hexanol (6:1, v/v) to dried, preweighted amounts of Brij30, Triton X-100, SDS, CTAB, Brij58 (to give total concentration of 133, 77, 22, 11 and 7 mM, respectively), and further injecting the required volume of the water buffer. The mixtures were shaken until being optically clear (5–10 s).

2.3. Turbidity and absorption spectra measurements

The absorption spectra of reverse microemulsions and Flu-4-dUTP were recorded on a Hitachi U-0080D photodiode array UV–vis spectrophotometer by using 1-cm path length cells. The reverse micelle pseudo-absorbance signal, *A*, read in an absorption spectrophotometer is related to turbidity by: $\tau = 2.303A$.

2.4. DNA polymerase assays in reverse microemulsions

The final mixtures (0.1 ml) contained 20 mM Tris, 2.5 mM $MgCl_2$, 1 mM dithiothreitol, 10 μM dNTP, 0.5–3.0 nM M13mp19 ssDNA, and 0.5–3.0 nM 5′-[^{32}P]GGCGATTAAGTTGGG primer (specific activity of 1 Ci/μmol). The reaction mixtures containing the substrates were introduced into the organic system as water solutions. Microemulsions were formed by vigorous stirring in vortex. After formation of the microemulsions, the DNA polymerase (30–100 nM) in the water buffer was introduced to start the reaction. After being vigorously stirred, the mixtures were incubated at 0–65 °C for 30 min. The nucleotide material was precipitated with 1 ml of 2% $LiClO_4$ in acetone at 0 °C, washed with cold acetone (1 ml), dried, and dissolved in 7 μl of 95% formamide containing 0.5% bromophenol blue as a marker dye. Reaction products were separated by electrophoresis on 20% polyacrylamide gels in 7 M urea. The gels were dried and subjected to autoradiography and/or phosphoimaging for quantification using Molecular Imager FX (Bio-Rad) and software (Quantity One).

2.5. DNA polymerase assays in the water buffer

Reaction mixtures (10 μl) contained 50 mM Tris–HCl, pH 8.6, 10 mM $MgCl_2$, 50 mM KCl, 1 mM dithiothreitol, 100 μM dNTP, 5–30 nM M13mp19 ssDNA, 5–30 nM 5′-[^{32}P]GGCGATTAAGTTGGG primer (specific activity of 1 Ci/μmol). Reactions were initiated by adding the enzyme (0.1–1 μM). The mixtures were incubated at 0–65 °C for 30 min. The nucleotide material was precipitated with 1 ml of 2% $LiClO_4$ in acetone at 0 °C, washed with cold acetone (1 ml), dried, and dissolved in 7 μl of 95% formamide containing 0.5% bromophenol blue as a marker dye. Reaction products were separated and visualized as described above.

3. Results and discussion

3.1. The exploration of reverse micellar microenvironment

In our previous papers we have shown that DNA polymerases does not work in ionic reverse microemulsions, are slightly active in nonionic microenvironment and are the most active in the microemulsions composed of mixed ionic and nonionic surfactant [14,15]. The reverse microemulsion (also called water-in-oil microemulsion or reverse micelle) consists of an aqueous microdomain facing the polar heads of surfactant that surrounds this core interacting with the bulk organic solvent, through the hydrophobic

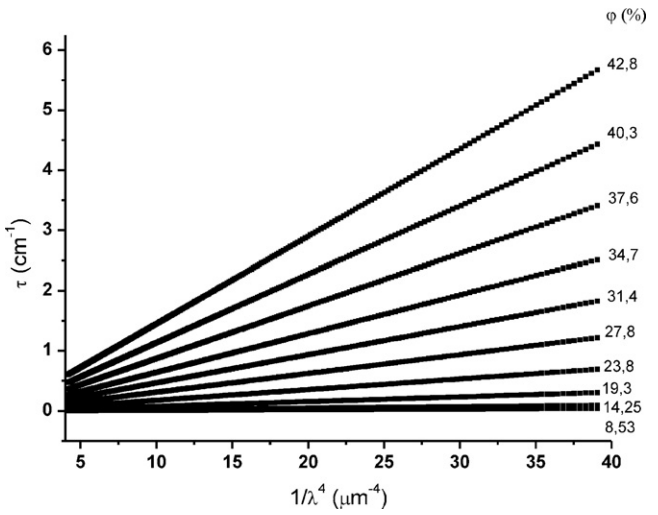

Fig. 1. The dependence of reverse micelle turbidity (τ) on wavelength (λ) of the light at various water content (φ).

chains. The reverse microemulsion definitions do not include some important properties, concerning the behavior of this system. One is the fact that the microemulsions are dynamical entities, which can exchange their constituents including water, surfactant, or other contents. The mixed multi-component systems provide an environment in which the different type of interaction can be realized, including hydrophobic, van der Waals, electrostatic and hydrogen bond interaction. The fluidity of the microemulsions is permanent since no covalent chemical bonds are formed, and, therefore, the structure and dynamics of reverse microemulsions is expected to be modified by solubilized proteins and nucleic acids. In fact, the enzyme and DNA can create cell-like environment by rearrangement of different types of surfactants at surface. On the other hand, the microemulsion system can change the structure of protein and DNA. Under the circumstances, the true criterion of native-like environment for given enzyme is its high-catalytic efficiency. Compared to reverse micelles created from ionic surfactants, there exists substantially less information about nonionic reverse micelles and, especially, about mixed multi-component systems. Therefore, we have investigated some physicochemical properties of the reversed microemulsions used in this work.

The turbidity measurements are the simple method to estimate the size of the scattering particles. Fig. 1 shows that the turbidity of reverse microemulsions is inversely proportional to the fourth power of the electromagnetic wavelength as predicted by classical light scattering theory (Rayleigh scattering). Rayleigh theory applies to small particles with radii less than 1/10 the wavelength of the radiation and predicts $\tau \sim r^6/\lambda^4$, where τ is the turbidity, *r* the radius of the scattering particles, and λ is the electromagnetic wavelength. Therefore, our results indicate that the reverse microemulsions are smaller that 25 nm, and their sizes are decreased with a decrease in the water content. The fitting of experimental points by a linear function of $1/\lambda^4$ in the range from 400 to 700 nm gave the correlation coefficient $R = 1$ (standard deviation S.D. = 10^{-15}), according to the Rayleigh light scattering theory. This result indicates the relatively low polydispersity of water droplet sizes as well.

The physical characteristics of the water in the reverse micellar medium are strongly depending on the molar ratio of water to surfactant and on the nature of the surfactant. Experimental studies of water pool confined in reverse micelles revealed a substantial decrease of polarity and a dramatic slowing down of the rotational relaxation of water molecules [18–23]. We investigate the microenvironment in our reverse microemulsions using fluorescein