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Interactions of the DNA polymerase X from African Swine Fever Virus with the ssDNA. Properties of the total DNA-binding site and the strong DNA-binding subsite ☆

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ABSTRACT

Interactions of the polymerase X from the African Swine Fever Virus with the ssDNA have been studied, using quantitative fluorescence titration and fluorescence resonance energy transfer techniques. The primary DNA-binding subsite of the enzyme, independent of the DNA conformation, is located on the C-terminal domain. Association of the bound DNA with the catalytic N-terminal domain finalizes the engagement of the total DNA-binding site of the enzyme and induces a large topological change in the structure of the bound ssDNA. The free energy of binding includes a conformational transition of the protein. Large positive enthalpy changes accompanying the ASFV pol X–ssDNA association indicate that conformational changes of the complex are induced by the engagement of the N-terminal domain. The enthalpy changes are offset by large entropy changes accompanying the DNA binding to the C-terminal domain and the total DNA-binding site, predominantly resulting from the release of water molecules.

1. Introduction

The African Swine Fever Virus (ASFV) has been the subject of intensive studies as the etiological agent responsible for acute hemorrhagic fever in domestic pigs [1–5]. The ASFV polymerase X is one of the proteins coded by the virus genome, which shows significant functional similarities to DNA-repair polymerases that include distributive DNA synthesis on template-primer DNA substrates and efficient filling of single nucleotide gaps [4–6]. These functional activities and the fact that the ASFV genome codes for the replicative polymerase and several enzymes involved in the base excision repair (BER) pathway, indicate that the major role of the ASFV pol X is to repair the viral DNA damaged by the host reaction to the viral infection [4,5].

The ASFV pol X is currently the smallest known DNA polymerase with a molecular weight of ~20,000, whose structure has been determined by the NMR method [7,8]. The enzyme is built of two domains, the N-terminal domain, which includes the first 105 amino acids from the N-terminus of the protein and the C-terminal domain, comprising the remaining 69 amino acid residues, as depicted in Fig. 1a. The active site of the DNA synthesis with the triad of invariant aspartate residues is located in the N-terminal domain (Fig. 1b). Moreover, the N-terminal domain contains a positively charged helix, αC. Similarly, the C-terminal domain contains a highly positively charged helix, αE (Fig. 1a) [7,8]. Both helices were implicated in the enzyme interactions with the DNA[7,8,11]. The simplified structure of the ASFV pol X makes the enzyme an excellent model system for the examination of the properties of a DNA repair polymerase.

Quantitative thermodynamic studies provided the first indication of the intricate interactions of the ASFV pol X with the ssDNA, in spite of the simplified structure of the protein [9–11]. The total site-size of the enzyme–ssDNA complex, *i.e.*, the maximum number of nucleotides occluded by the polymerase, is 16 ± 1 nucleotides. The total DNA-binding site has a heterogeneous structure, containing the strong ssDNA-binding subsite, which encompasses only 7 ± 1 nucleotides. Moreover, the subsite shows a significant preference for the dsDNA conformation over the ssDNA [10]. Fluorescence resonance energy transfer (FRET) data showed that the dsDNA binds to the C-terminal domain of the enzyme, a strong indication that the strong ssDNA-binding subsite is located on the domain (Fig. 1a and b) [10]. The enzyme engages the weak DNA-binding subsite in interactions only with the longer ssDNA oligomers. However, the efficiency of this engagement depends on the length of the ssDNA [9].

Although significant progress has been achieved in elucidating the ASFV pol X - DNA interactions, the nature of these interactions is still

Abbreviations: ASFV, African Swine Fever Virus; DTT, dithiothreitol; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; εA, etheno-adenosine; CP, 7-Diethylamino-3-(4′-maleimidylphenyl)-4-methylcoumarin; MCT method, Macromolecular Competition Titration Method; BER, base excision repair; FRET, fluorescence resonance energy transfer.

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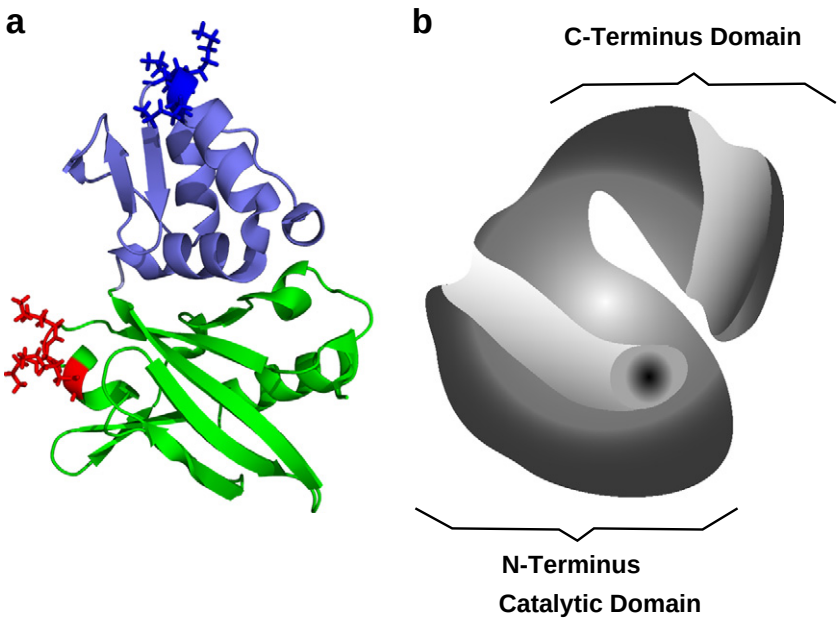

Fig. 1. a. Three-dimensional structure of the ASFV pol X obtained by NMR analyses [7,8]. The first 105 amino acid residues from the N-terminus of the protein (green) constitute the palm domain of the enzyme, while the remaining 69 amino acid residues (red) form the C-terminal domain. The lysine residues, 59, 60, and 63 contained in the αC helix of the palm domain and the lysine residues 131, 132, and 133 in the αE helix in the C-terminal domain, are marked in red and blue colors, respectively. b. Schematic representation of the ASFV pol X with the marked the strong DNA-binding subsite on the C-terminal and the weak DNA-binding subsite on the N-terminal domain, respectively. Both subsites form the total DNA-binding site of the enzyme [9,10]. The dark oval represents the location of the active site of the polymerase.

not completely understood. For instance, both the N-terminal and the C-terminal domains contain similar positively charged helices (Fig. 1a). Yet, the affinity of the strong DNA-binding subsite on the C-terminal domain is orders of magnitude higher than the affinity of the weak subsite [9–11]. Moreover, the reason for the significantly higher dsDNA-affinity of the strong subsite, as compared to its ssDNA-affinity, is unknown [10]. The NMR structure of the enzyme does not provide any clue about these different functional behaviors. While the release of only ~1 ion accompanies the exclusive ssDNA binding to the strong subsite, the release of ~6 ions occurs upon the engagement of the total DNA-binding site. These data would suggest that ionic interactions dominate the association with the weak subsite, but not the binding to the strong subsite on the C-terminal domain, though both subsites have the same number of exposed lysine residues in the presumed binding areas [7,8]. Furthermore, the structure of the DNA, exclusively bound to the strong subsite on the C-terminal domain, is characterized by a significantly larger separation and immobilization of the bound DNA bases than the structure of the bound longer DNAs, which encompass the total DNA-binding site of the polymerase.

Interactions of a DNA polymerase with the nucleic acid play a fundamental role in the functioning of the enzyme, including fidelity of the DNA synthesis, as the polymerase complex with the DNA constitutes the binding and recognition site for dNTPs [12–14]. Moreover, in the case of a DNA repair polymerase, elucidation of the enzyme - DNA interactions is of paramount importance for understanding the recognition mechanism of the damaged DNA [7–14]. In this communication, we examine the energetics of interactions of the total DNA-binding site and the strong DNA-binding subsite of the ASFV pol X with the ssDNA. The intrinsic affinity of the total site is not a simple sum of contributions from the strong and weak DNA-binding subsites. The protein–ssDNA complex undergoes a conformational transition induced by the nucleic acid interactions with the N-terminal domain. The intrinsic affinities of the strong subsite and the total DNA-binding site are predominantly driven by large entropy changes resulting from the release of water molecules from both the strong and weak DNA-binding subsites of the protein.

2. Materials and methods

2.1. Reagents and buffers

All chemicals were reagent grade. All solutions were made with distilled and deionized >18 MΩ (Milli-Q Plus) water. Standard buffer C is 10 mM sodium cacodylate adjusted to pH 7.0 with HCl, 50 mM NaCl, 1 mM $MgCl_2$, 1 mM DTT, and 10% glycerol (w/v).

2.2. The ASFV pol X

The plasmid harboring the gene of the ASFV pol X was a generous gift of Dr. Maria L. Salas (Universidad Autonoma, Madrid, Spain). Isolation and purification of the protein was performed, with slight modifications, as described [8–11]. The concentration of the protein was spectrophotometrically determined using the extinction coefficient $\varepsilon_{280} = 1.656 \times 10^5\ cm^{-1}\ M^{-1}$, obtained with the approach based on Edelhoch's method [15,16].

2.3. Nucleic acids

All unmodified and modified ssDNA oligomers were purchased from Midland Certified Reagents (Midland, Texas). The labeled ssDNA oligomer, $dT(pT)_{19}$, contains a fluorescent marker, fluorescein (Fl), attached at the 5′ end through phosphoramidate chemistry, and/or the coumarin derivative (CP), attached through the 6-carbon linker at the 3′ end. The labeled ssDNA oligomers are referred to as: 5′-Fl-$dT(pT)_{19}$, $dT(pT)_{19}$-CP-3′, and 5′-Fl-$dT(pT)_{19}$-CP-3′. The etheno-derivatives of homo-adenosine oligomers were obtained by modification with chloroacetaldehyde as previously described by us [17–23]. Concentrations of all ssDNA oligomers and the degree of labeling with the fluorescent markers, γ, have been spectrophotometrically determined [17–21,24]. The labeling with coumarin and fluorescein markers was complete in all examined oligomers and characterized by $\gamma = 1 \pm 0.03$. The dsDNA 10-mer has been built of the oligomer, ACGAGCCTGC, and the complementary strand. The integrity of the dsDNA oligomers has