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DNA Polymerase X From African Swine Fever Virus: Quantitative Analysis of the Enzyme–ssDNA Interactions and the Functional Structure of the Complex

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Interactions of polymerase X from African swine fever virus with single-stranded DNA (ssDNA) have been studied, using quantitative fluorescence titration and analytical ultracentrifugation techniques. Experiments were performed with a fluorescent etheno-derivative of ssDNA oligomers. Studies of unmodified ssDNA oligomers were carried out using the competition titration method. The total site-size of the pol X–ssDNA complex is 16($\pm$1) nucleotide residues. The large total ssDNA-binding site has a complex heterogeneous structure. It contains the proper ssDNA-binding site that encompasses only 7($\pm$1) residues. As the length of the ssDNA increases, the enzyme engages an additional binding area in interactions with the DNA, at a distance of ~7–8 nucleotides from the proper site, which is located asymmetrically within the polymerase molecule. As a result, the net ion release accompanying the interactions with the DNA, increases from ~1 for the proper DNA-binding site to ~6 for the total DNA-binding site. Unlike in the case of the mammalian polymerase β that belongs to the same polymerase X family, the DNA-binding areas within the total DNA-binding site of pol X are not autonomous. Consequently, the enzyme does not form different binding modes with different numbers of occluded nucleotide residues, although the interacting areas are structurally separated. The statistical thermodynamic model that accounts for the engagement of the proper and the total DNA-binding site in interactions with the DNA provides an excellent description of the binding process. Pol X binds the ssDNA without detectable cooperativity and with very modest base specificity.

Keywords: polymerases; DNA replication; protein–DNA interactions; motor proteins; fluorescence titrations

Introduction

The African swine fever virus (ASFV) is the etiological agent responsible for a highly lethal disease of domestic pigs.[1–7] The DNA genome of the virus encodes two DNA polymerases, the virus replicative polymerase, belonging to the eukaryotic family B enzymes and another polymerase (pol), a member of the pol X family referred to as ASFV pol X.[1,6,7] The pol X family comprises several polymerases with different and specialized functions in the cell.[6–9] The most intensively studied enzyme of this family is the mammalian pol β, an enzyme of ~39 kDa, which plays a very specialized function in cell DNA repair machinery.[9–21] pol β shows a typical polymerase fold, a thumb, a palm, and fingers, due to its resemblance to the human hand.[10] pol β contains an additional, N-terminal 8 kDa domain, not present in the DNA replicative polymerases. The domain is the primary DNA-

Abbreviations used: ASFV, African swine fever virus; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; εA, etheno-adenosine; MCT method, macromolecular competition titration method; BER, base excision repair; pol, polymerase.

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binding site of the enzyme with a significant degree of functional autonomy and spatial separation from the rest of the enzyme molecule.[15–21]

On the other hand, NMR studies of ASFV pol X revealed a structure very different from pol β and other DNA replicative or repair polymerases.[22,23] The enzyme is built only of the palm domain, which includes the first 105 amino acid residues from the N terminus of the protein and the C-terminal domain, built of the remaining 69 amino acid residues, as depicted in Figure 1.[22] With a molecular mass of ~20,000, ASFV pol X is the smallest, currently known, DNA polymerase. The palm domain contains a triad of invariant aspartate residues involved in divalent cation binding and the catalysis of the DNA synthesis by the DNA polymerases.[22,23] Moreover, the palm domain has a subdomain fold, unique for ASFV pol X, that comprises a positively charged helix, αC, positioned at the surface of the domain. Similarly, the C-terminal domain contains a highly positively charged helix, αE, which does not have its counterpart in pol β.[10,22,23] Due to these structural features and qualitative NMR data, both the palm and the C-terminal domain were implicated in DNA binding, although the structure of the co-complex has not been determined.[22,23]

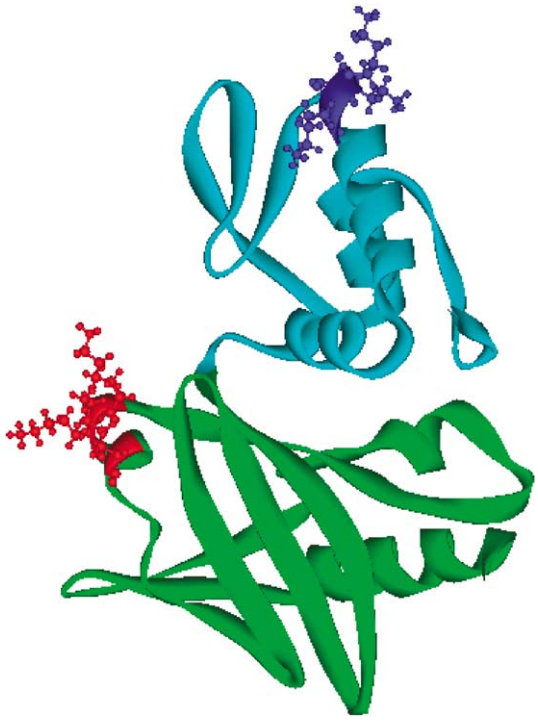

Figure 1. Three-dimensional structure of ASFV pol X obtained by NMR studies.[22,23] The palm domain, the first 105 amino acid residues from the N terminus of the protein, and the C-terminal domain, the remaining 69 amino acid residues, are marked in green and turquoise, respectively. Also, the lysine residues, 59, 60, and 63 of the palm domain, and the lysine residues 131, 132, and 133 contained in the αC helix, the αE helix of the palm and the C-terminal domain, respectively, are selected and marked in red and blue, respectively.

Besides the primary structure homology with the members of the polymerase X family, ASFV pol X shows striking functional similarities to the β-type polymerases that include template-directed DNA synthesis, distributive DNA synthesis on template-primer DNA substrates, increased processivity on the gapped DNAs with the ssDNA gap shorter than four nucleotide residues, and particularly efficient filling of the single-nucleotide gaps.[6,7,24] Such a spectrum of functional activities strongly suggests that ASFV pol X is involved in the repair processes of the viral DNA. This notion is further supported by the fact that the ASFV genome codes for another strictly replicative DNA polymerase, belonging to the B family of polymerases (see above), and several enzymes that perform most of the base excision repair (BER) pathway.[4–7] In other words, pol X is a part of the ASFV DNA repair apparatus geared to repair the viral DNA, which has been damaged by the host reaction to the viral infection.[6,7,22,23]

Interactions of a polymerase with the DNA play a vital role in the functioning of the enzyme.[25–27] Moreover, they are increasingly recognized as one of the major elements, that determine the degree of fidelity of the DNA synthesis, as the polymerase complex with the DNA constitutes the binding and recognition site for the dNTP. Furthermore, in the case of a DNA repair polymerase, elucidation of the enzyme interactions with the nucleic acid is of paramount importance for understanding the recognition mechanism of the damaged DNA by the enzyme. Thus, the mammalian pol β initiates the binding to the single-stranded (ss) or double-stranded (ds) DNA through its 8 kDa domain.[19,21,28–31] The autonomous nature of the DNA-binding subsite on the 8 kDa domain allows pol β to bind small areas of the DNA substrate without engaging the catalytic 31 kDa domain of the enzyme into interactions with the nucleic acid.[15–21,28–31] The subsequent association of the 31 kDa domain with the available DNA, follows this initial binding process.[28–31]

However, ASFV pol X does not possess the 8 kDa domain or an equivalent structural element, which would suggest a mechanism of binding similar to pol β (Figure 1). How a polymerase with such a simplified structure as ASFV pol X can still bind DNA with high affinity and recognize the damaged DNA structure is unknown. In spite of its paramount importance for understanding the DNA recognition process by the DNA repair polymerase, which is engaged in the viral defense mechanism against the host reaction to the infection, the direct and quantitative analyses of pol X interactions with the DNA has not yet been addressed. Such fundamental parameters of these interactions, like the site-size of the complex, i.e. number of nucleotide residues or base-pairs occluded by the enzyme in the complex, the functional structure of the DNA-binding site, intrinsic affinities, and cooperativity of the binding process are still unknown.

Here, we describe quantitative analyses of interactions of ASFV pol X with the ssDNA.