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Role of the catalytic metal during polymerization by DNA polymerase lambda

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ABSTRACT

The incorporation of dNMPs into DNA by polymerases involves a phosphoryl transfer reaction hypothesized to require two divalent metal ions. Here we investigate this hypothesis using as a model human DNA polymerase λ (Pol λ), an enzyme suggested to be activated *in vivo* by manganese. We report the crystal structures of four complexes of human Pol λ. In a 1.9 Å structure of Pol λ containing a 3′-OH and the non-hydrolyzable analog dUpnpp, a non-catalytic Na^+ ion occupies the site for metal A and the ribose of the primer-terminal nucleotide is found in a conformation that positions the acceptor 3′-OH out of line with the α-phosphate and the bridging oxygen of the pyrophosphate leaving group. Soaking this crystal in $MnCl_2$ yielded a 2.0 Å structure with Mn^{2+} occupying the site for metal A. In the presence of Mn^{2+}, the conformation of the ribose is C3′-endo and the 3′-oxygen is in line with the leaving oxygen, at a distance from the phosphorus atom of the α-phosphate (3.69 Å) consistent with and supporting a catalytic mechanism involving two divalent metal ions. Finally, soaking with $MnCl_2$ converted a pre-catalytic Pol λ/Na^+ complex with unreacted dCTP in the active site into a product complex via catalysis in the crystal. These data provide pre- and post-transition state information and outline in a single crystal the pathway for the phosphoryl transfer reaction carried out by DNA polymerases.

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1. Introduction

DNA polymerases are crucial for a large number of DNA transactions [1–3]. They are essential for genome replication, they perform gap-filling synthesis in several DNA repair pathways, they perform translesion DNA synthesis and they contribute to somatic hypermutation of immunoglobulin genes. These roles all depend on the simple reaction that they catalyze, the incorporation of a nucleoside monophosphate onto the 3′ terminus of a DNA chain and the release of pyrophosphate. This reaction consists of a nucleophilic substitution in which the 3′-oxygen of the primer-terminal nucleotide donates an electron pair to form a phosphorus–oxygen bond with the phosphorus of the α-phosphate of the incoming deoxynucleoside triphosphate. Concomitantly, the bond between this phosphorus atom and the oxygen bridging the α- and β-phosphates is broken, resulting in the release of pyrophosphate. By analogy to the mechanism proposed for a 3′-exonuclease reaction

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[4], a mechanism for this reaction was proposed in 1993 [5] and remains current today. DNA polymerases were proposed to catalyze the reaction with the help of two divalent metal ions, which *in vivo* are generally believed to be Mg^{2+}, but sometimes may possibly be Mn^{2+} [6]. One metal (A, or catalytic) is suggested to serve a catalytic role by lowering the pK_a of the 3′-OH at the primer terminus and/or by positioning it in a proper geometry for catalysis. The other metal (B, or dNTP-binding) coordinates the triphosphate moiety of the incoming dNTP, facilitating dNTP binding and subsequent release of pyrophosphate.

The two metal ion model is consistent with the stereochemistry of the polymerization reaction [7] and with a number of structural studies of DNA polymerases. Several groups have reported DNA polymerase crystal structures with metal ions present. However, in some cases only one metal is observed, in other cases the metals are not physiological (Ca^{2+}), and in still other cases the identity of the metal is uncertain. Moreover, the geometry at the active site is not usually consistent with an in line nucleophilic attack by the 3′-oxygen. In fact, the 3′-oxygen is often missing because a favorite method to trap pre-catalytic complexes of DNA polymerases is to employ a dideoxy-terminated primer (i.e., missing the 3′-OH). The exception is a recent study by Wilson and co-workers [8] describing the structure of a pre-catalytic complex of DNA polymerase β bound to gapped DNA, with a non-hydrolyzable nucleotide analog and two divalent metal ions bound at the active site. This structure (discussed further below), and the present study of DNA polymerase λ, strongly supports the proposal that two divalent metal ions are required for catalysis.

Like Pol β, Pol λ is a family X polymerase that fills short gaps during DNA repair [9]. Pol λ and Pol β have similar properties, although Pol λ appears to display a larger flexibility with respect to the substrates on which it can polymerize [10]. This flexibility was first suggested by the observation that Pol λ generates deletion errors at an extremely high rate [11], and is now thought to be a feature of the enzyme that facilitates its role *in vivo*. The phenotypes of mice deficient in this polymerase indicate that Pol λ plays a role in the V(D)J process of antigen gene diversification [12], and several reports implicate Pol λ in base excision repair [13–15] and the repair of double-strand breaks through the non-homologous DNA end-joining pathway [16–18]. It is thus interesting to examine the details of the polymerization reaction as catalyzed by Pol λ in order to understand whether specific catalytic features can account for these special properties.

The structure of the catalytic core of human Pol λ has been extensively characterized [9,10,19–22]. Although structurally similar to Pol β, Pol λ displays some unique structural differences. For instance, upon dNTP binding, Pol β undergoes a large-scale conformational change in which its thumb subdomain rotates by about 25° to assemble a catalytic conformation. By contrast, comparison of pre- and post-catalytic complexes of Pol λ suggests that no such subdomain motion takes place throughout the Pol λ catalytic cycle [21]. Instead, a catalytic conformation is achieved through the movement of the DNA template strand and the side chains of a few active site residues (see Fig. 2A in [21]). Moreover, several structures of Pol λ bound to misaligned and/or mismatched substrates illustrate the substrate flexibility of the enzyme and have identified some of the structural elements that are thought to facilitate this flexibility [10,22].

A number of structures of Pol λ bound to a normal DNA duplex have provided insights into the reaction mechanism. However, to date no pre-catalytic structure of Pol λ containing all the atoms involved in the reaction has been obtained, and the role, or even the presence, of the catalytic metal ion has been uncertain [21]. Here, we provide additional structures of Pol λ that include the essential catalytic atoms. These structures extend the characterization of the Pol λ reaction mechanism and strongly support the catalytic relevance of previous structures.

2. Materials and methods

2.1. *Proteins and nucleotides*

The human Pol λ catalytic core with a C543A mutation to eliminate intermolecular disulfide bond formation was expressed and purified as described [21]. Oligonucleotides Td (5′-CGGCAGTACTG), Pd (5′-CAGTAC), and DT (5′-GCCG), for the dUpnpp structures and Tc (5′-CGGCGGTACTG), Pc (5′-CAGTAC), and DT, for the dCTP structures were from Oligos Etc. dCTP was from GE Healthcare and dUpnpp was from Jena Bioscience.

2.2. *Protein crystallization and structure determination*

Crystals were grown essentially as described [21]. Briefly, crystals were formed using the hanging drop method by mixing 2 μl of the protein solution containing DNA and the appropriate nucleotide (10 mM) with 2 μl of reservoir solution containing 10–20% 2-propanol, 0.2 M sodium citrate, and 0.1 M sodium cacodylate (pH 5.5). The protein solution contains 100 mM NaCl and 1 mM $MgCl_2$. The crystals that did not require soaking were transferred in five steps to a solution containing 19% 2-propanol (dCTP) or 21% (dUpnpp), 0.2 M sodium citrate, 0.1 M sodium cacodylate (pH 5.5), 100 mM NaCl, 1 mM $MgCl_2$, and 25% (w/v) ethylene glycol. Data sets for crystals containing Mn^{2+} ions were made possible by soaking crystals for 1 h in a solution containing 19% (dCTP soak) or 21% (dUpnpp soak) 2-propanol, 0.1 M sodium cacodylate (pH 5.5), 0.2 M sodium citrate, and 200 mM $MnCl_2$. Subsequently, they were soaked three times for 45′ into a solution containing 19% (dCTP soak) or 21% (dUpnpp soak) 2-propanol, 0.1 M sodium cacodylate (pH 5.5), 1 mM sodium pyrophosphate, 300 mN NaCl, and 20 mM $MnCl_2$. After soaking, the crystals were transferred in five steps to a solution containing 0.1 M sodium cacodylate (pH 5.5), 0.3 M NaCl, 20 mM $MnCl_2$, 1 mM sodium pyrophosphate, 19% (dCTP soak) or 21% (dUpnpp) 2-propanol, and 25% (w/v) ethylene glycol. All crystals were frozen in liquid nitrogen and then mounted on a goniometer in a cold stream of nitrogen at −178 °C for data collection. Data were collected on a Rigaku 007HF generator equipped with Varimax HF mirrors and a Saturn 92 detector. All data were processed using the HKL2000 software [23].