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Structural Insights into Yeast DNA Polymerase δ by Small Angle X-ray Scattering (SAXS)

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Summary

DNA polymerase δ (Pol δ) is a multi-subunit polymerase that plays an indispensable role in replication from yeast to humans. Pol δ from *S. cerevisiae* is composed of three subunits, Pol3, Pol31 and Pol32. Despite the elucidation of structures and models of the individual subunits (or portions, thereof) the nature of their assembly remains unclear. We present here a small-angle X-ray scattering (SAXS) analysis of a yeast Pol δ complex (Pol δ_T) composed of Pol3, Pol31, and Pol32N (amino acids 1–103 of Pol32). From the SAXS global parameters and reconstructed envelopes we show that Pol δ_T adopts an elongated conformation with a radius of gyration (R_G) of $\sim 52\text{\AA}$ and a maximal dimension of $\sim 190\text{\AA}$. We also propose an orientation for the accessory Pol31-Pol32N subunits relative to the Pol3 catalytic core that best agrees with the experimental scattering profile. The analysis also points to significant conformational variability that may allow Pol δ to better coordinate its action with other proteins at the replication fork.

The eukaryotic DNA replication machinery is conserved from yeast to humans and requires the actions of numerous proteins. DNA replication in eukaryotes is carried out by the multi-subunit polymerases (Pols) α , δ and ϵ 1–4. Briefly, Pol α primes the Okazaki fragments on the lagging strand, which are then elongated by Pol δ . Pol ϵ is believed to be the leading strand polymerase, though Pol δ can also fulfill that role. Thus, the replication of a large portion of the eukaryotic genome depends on Pol δ , which synthesizes not only the lagging strand but also contributes to the synthesis of the leading strand 1–4.

Pol δ from *S. cerevisiae* consists of three subunits, Pol3 (125 kDa), Pol31 (55 kDa) and Pol32 (40kDa). Pol3, a member of the B-family of Pols, is the catalytic subunit of the holoenzyme, encoding both the polymerase and the 3' to 5' exonuclease proofreading functions 5–7. Mutations in either the polymerase or the exonuclease domain of Pol δ that lower the fidelity of Pol δ cause tumors in mice and humans 8–12. Pol3 forms a stable complex with Pol31 (referred to as Pol δ^*), to which Pol32 is then tethered via interactions with Pol31 13–15. Thus, Pol31 bridges Pol3 and Pol32, and it interacts with the second of the two C-terminal zinc finger

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modules in Pol3¹⁶. Pol3 and Pol31 are essential for growth, wherein disruption of the *Pol3* and *Pol31* genes in yeast causes cell inviability^{5–7; 13; 17}. Pol32 is dispensable, though deletion mutants of Pol32 show defects in DNA replication, DNA repair, and mutagenesis¹³.

We have recently determined the structure of the yeast Pol3 catalytic core¹⁸, which reveals a distance of ~45Å between the polymerase and the exonuclease active sites. The structure of a complex between the human orthologs of Pol31 (p50) and the N-terminal portion of Pol32 (p66N) has also been determined¹⁹. However, the proper functioning of Polδ *in vivo* requires a complex of all three subunits, but there is little or no structural information on the holoenzyme. Although, low resolution EM structures for a yeast Polε holoenzyme and a yeast Polα heterodimer have become available^{20; 21}, for Polδ, knowledge about its conformation in solution is limited to analytical ultracentrifugation studies that suggest an extremely elongated shaped complex that give rise to a “dimer like” behavior on a gel filtration column¹⁵.

Small angle X-ray scattering (SAXS) is an efficient method for investigating dynamic complexes that are resistant to crystallography and NMR: providing low resolution information about both shape and flexibility^{22–24}. We present here SAXS analysis of a yeast Polδ complex composed of Pol3-Pol31-Pol32N (amino acids 1–103) subunits, which we refer to as Polδ_T. Our results indicate that Polδ_T is a monomeric enzyme that consists of a globular Pol3 catalytic core linked to an elongated tail structure composed of the Pol31-Pol32N subunits. The overall shape of the complex is similar to that obtained for yeast Polα by EM²¹. Our analysis also suggests conformational variability in how the Pol31-Pol32N subunits are oriented with respect to the Pol3 core and which may help to coordinate Polδ's action with other proteins at the replication fork.

To define the overall conformation of Polδ_T (including its functional flexibility) we measured SAXS data for Polδ_T at two different concentrations (0.5 and 2 mg/mL) (Fig. 1a). The SAXS data were merged to define the entire scattering profile based on the standard protocols^{22; 23}. Different protein concentrations were also tested for aggregation and examined by Guinier plots²⁵. The linearity in the Guinier plot (Fig. 1a-inset) was examined in the region $R_g \cdot q < 1.5$ and indicated that the Polδ_T sample is not aggregated and has an estimated R_g value of 52Å. To gain further information on the geometry of Polδ_T, we calculated the pair-distance distribution function, $P(r)$ (Fig. 1b), from the SAXS curve (Fig. 1a). The $P(r)$ function provides a real space interpretation of the scattering data and revealed peak-maximum at 47Å. This value corresponds to the most frequently occupied interatomic distances in Polδ_T. Furthermore, the R_g value of 53Å derived from the $P(r)$ is likewise internally consistent with the R_g calculated from the Guinier approximation described above. Interestingly, the profile of the $P(r)$ function exhibited a long tail with a maximal particle dimension, D_{max} , of ~190Å (Fig. 1b). The simplest interpretation of these features is that the intact Polδ_T protein is substantially elongated in solution. To test if partial dimerization is responsible for the experimentally observed elongation, we performed two experiments. First, we estimated the molecular weight of Polδ_T, using intensities at zero angles and three reference proteins²⁶ (Lysozyme, Bovine Serum Albumin, Glucose Isomerase), which showed that Polδ_T is a monomer in solution with estimated Mw_{saxs} = 180kDa (Fig. 1c), similar to the Mw = 190kDa estimated from sequence. Second, we visualized Polδ_T by native PAGE gel which confirmed the homogeneity of the sample (Fig. 1d).

To visualize the elongated shape(s) of Polδ_T predicted by its $P(r)$ function, we constructed SAXS envelopes using the program GASBOR²⁷ (Fig. 2a). 16 independent reconstructions were generated, superimposed and averaged using the software DAMAVER²⁸. Polδ_T appears to be comprised of a compact globular domain with a long and narrower tail that extends away from the globular domain. Based on our knowledge of the structure of the yeast Pol3 catalytic core and the Pol31-Pol32 homologs^{18; 19}, and the current biochemical information on the

subunit-subunit interactions in Pol δ ^{13–16}, we assigned the globular region of the envelope to Pol3 and the tail region to Pol31-Pol32N (with Pol32N directed away from Pol3) (Fig. 2a).

To further test the elongated character of Pol δ_T in solution, we used rigid body modeling. That is, a molecular dynamics (MD) simulation was used to probe the conformational distribution of Pol31-Pol32N that best matched the solution scattering data²⁴. We performed modeling with the known structures of the yeast Pol3 catalytic core, and a homology model for Pol31-Pol32N derived from the crystal structure of the human orthologs, p50-p66N. A model for the two zinc finger modules in Pol3 (amino acids 986–1097) was derived using the program Rosetta²⁹. Based on the crystal structure of the p50-p66N complex¹⁹, which shows the two subunits to be rigidly docked with $\sim 5398 \text{ \AA}^2$ of surface area buried at the interface, we reasoned that the interface of Pol31-Pol32N was also rigid and was therefore maintained as such during MD simulation. The hinge regions between the catalytic core of Pol3 and the two C-terminal zinc-finger modules were treated as flexible. MD simulation was used to generate several thousand models of Pol δ_T (Fig. 2b) with varying orientation of Pol31-Pol32N with respect to the Pol3 catalytic core, and their scattering profiles calculated by CRY SOL³⁰ and compared to the experimental scattering profile. The SAXS-validated best fitting model ($\chi^2 = 1.8$; Figs. 2a and b) shows Pol31-Pol32N extending away from the Pol3 core with D_{max} and R_G values of $\sim 150 \text{ \AA}$ and $47 \text{ \AA}$, respectively. Interestingly, there is no contact between the catalytic core of Pol3 and Pol32N, consistent with biochemical studies^{13–15} (Fig. 2c). Despite its overall similarity with the SAXS envelopes (Fig. 2a), the $P(r)$ for the best fit model is significantly different from the experimental $P(r)$ (Fig. 1b). The discrepancy between the model and experimental $P(r)$ indicates that a single conformer of Pol δ_T is not able to describe the conformational variability of the complex in solution. The sharper peak and longer tail in the experimental $P(r)$ (Fig. 1b) suggest conformational variability in how the Pol31-Pol32N “module” is oriented about the hinge regions between the catalytic core and the two C-terminal zinc-finger modules.

Taken together, we present here the first structural analysis of Pol δ_T by SAXS. The analysis reveals a complex that has an elongated shape in solution and suggests variability in the orientation of Pol31-Pol32N with respect to the catalytic core of Pol3. These features are similar to those determined for Pol ϵ and Pol α by EM analyses^{20; 21}, which reveal a flexible tail structure comprised of the accessory subunits (Dpb2, Dpb3, and Dpb4 in Pol ϵ and Pol12 in Pol α) linked to globular domain composed of the core of the catalytic subunit (Pol2 in Pol ϵ and Pol1 in Pol α). In Pol ϵ and Pol α , the accessory subunits have been suggested to accentuate DNA binding by the catalytic subunit^{20; 21}. Notably, the Pol31 subunit of Pol δ contains an OB-fold and an inactive phosphodiesterase domain that may play a role in increasing the affinity of Pol δ for DNA^{19;31}. Interestingly, the elongated shape of Pol δ_T places Pol32 $>50 \text{ \AA}$ from Pol3 catalytic core, where it can potentially interact with other proteins at the replication fork. However, the role of Pol32 in Pol δ function remains somewhat mysterious. Although, Pol32 carries a consensus PCNA interaction motif (QxxLxxFF) at its C-terminus, the motif is dispensable for Pol δ 's ability to bind PCNA on the DNA¹⁴. Also, based on secondary structure analysis, only the first ~ 100 residues of Pol32 (the segment in Pol32N) appear to be ordered, and the rest may only become ordered in association with PCNA (off the DNA) or other proteins. The variability in the orientation Pol31-Pol32N subunits with respect to the Pol3 catalytic core suggests that these subunits behave as an independent “module” that can freely access different regions of the replication fork. This flexibility may be important when there is a change in DNA direction as, for example, during a switch in the primer from the polymerase to the exonuclease active site during DNA synthesis. The elongated shape and flexibility of Pol δ may also facilitate the entry of translesion DNA synthesis (TLS) Pols to a stalled replication fork³². That is, it may allow one end of Pol δ to remain tethered to PCNA while the catalytic core moves “aside” to allow the entry of a TLS Pol to a stalled replication fork³³, for the bypass of a DNA lesion. Interestingly, Pol δ from *S. pombe* and humans harbors an

addition small fourth subunit that serves to stabilize the complex^{4, 34, 35}. From biochemical studies³⁵, this fourth subunit (p12) in human Pol δ appears to “cross-brace” the Pol3 and Pol31 orthologs, and also appears to interact with PCNA. In all, although the molecular details of how Pol δ interacts with PCNA and TLS Pols remain to be determined - SAXS offers an excellent method for investigating conformations of these dynamic complexes^{22; 23}.

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Figure 1.

Solution structural information from X-ray scattering data. The scattering data were collected at the ALS beamline 12.3.1 LBNL Berkeley, California and processed as previously described²². Wavelength (λ) and the sample-to-detector distances were set to 1.032 Å and 1.5 m, respectively, resulting in scattering vectors (q) ranging from 0.01 Å⁻¹ to 0.31 Å⁻¹ for $q = 4\pi \sin(\theta)/\lambda$, where 2θ is the scattering angle. **(a)** Experimental SAXS curves of the Pol δ_T (black line). Red line shows the theoretical scattering from the best-fit atomic models shown in Fig. 2c. (a-inset) Guinier plot for the experimental data with linear fit (pink line) in the limit $qR_G > 1.5$. The radius of gyration (R_G) was derived by the Guinier approximation $I(q) = I(0) \exp(-q^2 R_G^2/3)$ with the limits $qR_G < 1.3$. **(b)** Distance distribution functions of the Pol δ_T computed for experimental data (black line). $P(r)$ function for best-fit model is shown in red. The $P(r)$ functions are normalized to unity at their maxima. GNOM³⁶ was used to calculate the pair-distance distribution functions, $P(r)$, and define the maximum dimension of the macromolecule, D_{max} . **(c)** Molecular weight estimation obtained by calculating the normalized scattering intensity at zero angle ($q=0; I(0)$) relative to three standard, control proteins (Lysozyme, Bovin Serum Albumin, Glucose Isomerase). The molecular weights for standard (black circles) and Pol δ_T (green rectangle) are indicated. **(d)** Native PAGE gel of Pol δ_T

showing the homogeneity of the sample. Pol δ_T was expressed and purified as described previously for Pol δ ³³

Figure 2.

Solution structure modeling of Pol δ_T . **(a)** Five representative and average GASBOR²⁷ models of Pol δ_T are shown in volumetric format. Sixteen bead models obtained were averaged by the DAMAVER²⁸ program to construct the average model representing general structural features of each reconstruction. Right panel shows atomic best-fit model for Pol δ_T from rigid body modeling, superimposed on average SAXS envelope represented in mesh volumetric format. **(b)** Comparison of discrepancy with experimental data (χ^2) values for 10000 Pol δ_T models with their R_G values (black circles). The best-fit model is indicated by the red circle. **(c)** Two views of the best-fit model for Pol δ_T , obtained by conformational sampling using BILBOMD protocol²⁴, matching experimental data with $\chi^2 = 1.8$. Theoretical scattering and P(r) function profile for this model are shown in Figs 1a and 1b. The structure is shown in ribbon representation where the Pol3 domain is in blue with highlighted Zn1, Zn2 modules in cyan, Pol31 green and Pol32N in yellow. The drawing shows the proposed subunit organization of yeast Pol δ .