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Crystal structures of the Klenow fragment of *Thermus aquaticus* DNA polymerase I complexed with deoxyribonucleoside triphosphates

YING LI,¹ YONG KONG,^{1,2} SERGEY KOROLEV,¹ AND GABRIEL WAKSMAN¹

Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine,
660 South Euclid Avenue, Saint Louis, Missouri 63110

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Abstract

The crystal structures of the Klenow fragment of the *Thermus aquaticus* DNA polymerase I (Klenetaq1) complexed with four deoxyribonucleoside triphosphates (dNTP) have been determined to 2.5 Å resolution. The dNTPs bind adjacent to the O helix of Klenetaq1. The triphosphate moieties are at nearly identical positions in all four complexes and are anchored by three positively charged residues, Arg659, Lys663, and Arg587, and by two polar residues, His639 and Gln613. The configuration of the base moieties in the Klenetaq1/dNTP complexes demonstrates variability suggesting that dNTP binding is primarily determined by recognition and binding of the phosphate moiety. However, when superimposed on the Taq polymerase/blunt end DNA complex structure (Eom et al., 1996), two of the dNTP/Klenetaq1 structures demonstrate appropriate stacking of the nucleotide base with the 3' end of the DNA primer strand, suggesting that at least in these two binary complexes, the observed dNTP conformations are functionally relevant.

Keywords: crystal structure; DNA polymerase; dNTP; Klenetaq1

The DNA polymerase from *Thermus aquaticus* (Taq polymerase) belongs to the family of DNA polymerase I enzymes (DNA Pol I), which play a role in the repair of DNA lesions in prokaryotic organisms (Delarue et al., 1990; Kornberg & Baker, 1991). Taq polymerase is a single monomeric enzyme with a molecular weight of 93.9 kDa (Lawyer et al., 1993). The primary sequence of Taq polymerase is 38% identical to *Escherichia coli* DNA polymerase I (*E. coli* Pol I), an enzyme which has served as a model for the study of DNA replication by proteins of the DNA Pol I family (reviewed in Joyce & Steitz (1994)). Like other members of the Pol I family, Taq polymerase consists of three domains: an N-terminal domain (residues 1–290) which has a 5'-3' nuclease activity, an intervening domain (residues 291–419), and a C-terminal polymerase domain (residues 420–832), which is highly homologous in primary sequence and nearly identical in structure to the large domain of the Klenow fragment of *E. coli* Pol I (Kim et al., 1995).

While the intervening domain of *E. coli* Pol I supports a 3'-5' exonuclease activity, the equivalent region in Taq polymerase is

deprived of any catalytic function. The overall fold in that region is remarkably conserved in both proteins (Korolev et al., 1995). However, in Taq polymerase, helix A is missing. The loops connecting strand 4 and helix B, helix E and helix F are shorter in Taq polymerase than in *E. coli* Pol I. The residues of strand 2 (355, 357, and 358), helix C (424), and helix F (497, 501), which form the dTMP binding site in *E. coli* Pol I, are either missing (E357, T358) or replaced by hydrophobic residues: Asp355 in *E. coli* Pol I is replaced by Phe309 in Taq polymerase, and Asp424 is replaced by Leu356. As a result, this site in Taq Pol I is primarily hydrophobic and has lost the capability to function catalytically. Consequently, amplification by Taq polymerase is an error-prone process. Enzymes with N-terminal deletions, called Klenetaq1, show a reduced tendency toward errors (Barnes, 1992). The amplification of large fragments of DNA is achieved by combining Klenetaq1 with a low level of thermostable DNA polymerase exhibiting 3'-5' exonuclease activity (Barnes, 1994; Cheng et al., 1994).

Although DNA Pol I enzymes have been the subject of extensive crystallographic studies, the co-crystal structure of a member of this family bound to a duplex DNA close to the polymerase active site has only recently been determined (Eom et al., 1996). The crystal structure confirms early models of DNA replication in which the primer-template approaches the polymerase domain from the 3'-5' exonuclease side of the cleft (Joyce & Steitz, 1994). Although the DNA is about 2 Å away from a catalytically competent position, the blunt end of the duplex DNA is in the polymerase active site, packed against the O helix and the side chains of

Reprint requests to: Gabriel Waksman, Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, 660 South Euclid Avenue, Box 8231, Saint Louis, Missouri 63110; e-mail: waksman@gwiris1.wustl.edu.

¹These authors contributed equally to this work.

²Present address: Curagen, 555 Long Wharf Drive, New Haven, Connecticut 06511.

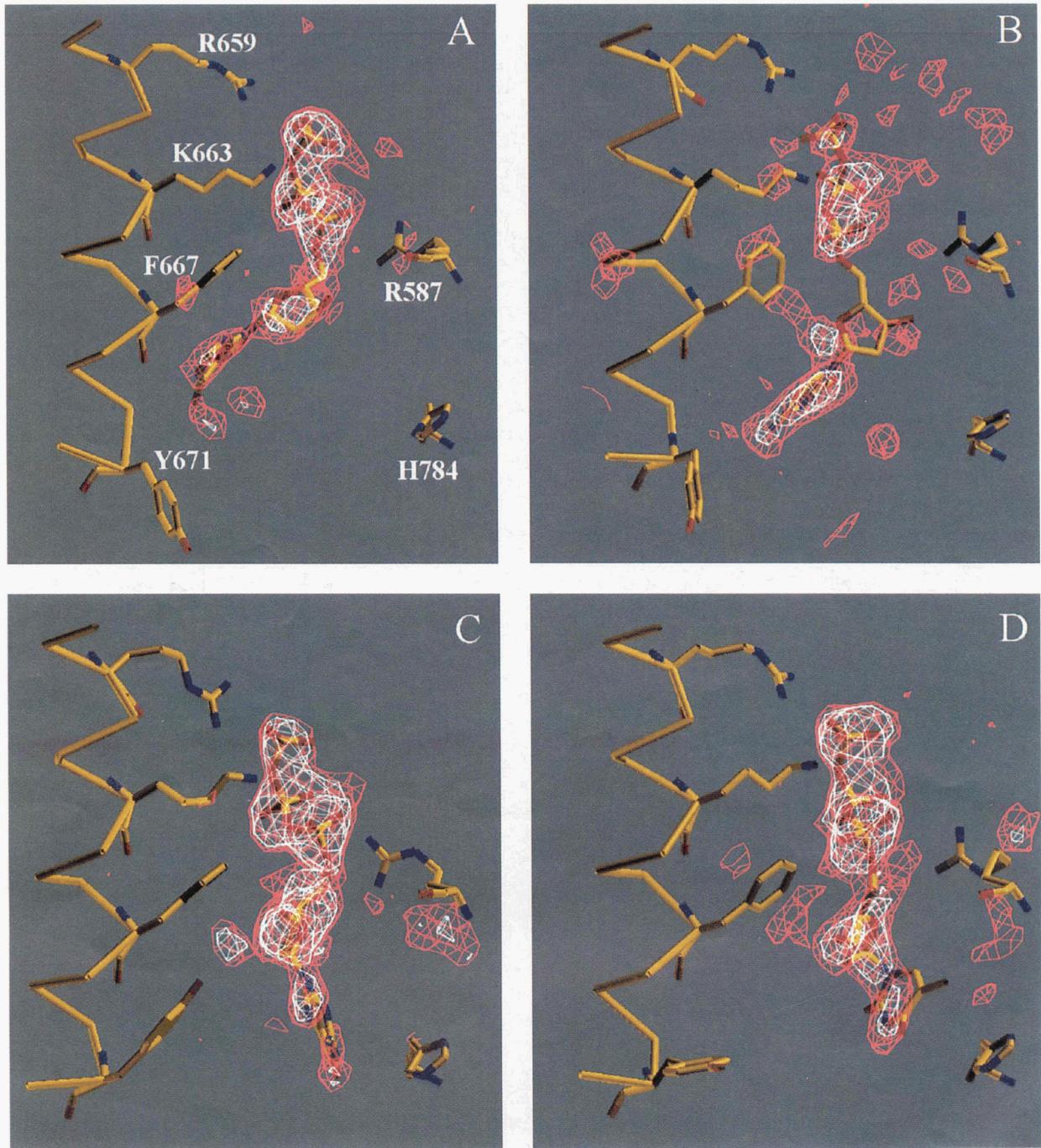


Fig. 1. $F_o - F_c$ map for the binary complexes of Klentaq1 with dNTP showing the electron density for the bound dNTP. The $F_o - F_c$ map calculated using the refined apo Klentaq1 model are contoured at 3σ (white) and 2σ (pink) levels for the binary complex of Klentaq1 with dATP (**A**), dCTP (**B**), dGTP (**C**), dTTP (**D**). F_o represent the observed structure factors for the binary complexes. The phosphate groups of the dNTP are placed in the strongest density (seen both at 2σ and 3σ level). The density for the sugar and base is less strong but still can be seen at a 2σ contour level, except for that of the sugar ring in the dCTP.

Asp785, Glu786, Asp610, which are conserved in all members of the Pol I family and form the catalytic core, are close to the 3' hydroxyl of the primer strand.

One important element in gaining an understanding of DNA duplication by DNA Pol I enzymes is the characterization of the interactions between enzyme and deoxynucleoside triphosphates

and the positioning of the primer/template binding site relative to the dNTP binding site. The structures of binary complexes of the Klenow fragment of *E. coli* Pol I with deoxynucleoside triphosphates or with pyrophosphate was determined to 3.9 Å resolution (Beese et al., 1993). These crystal structures show that the dNTPs bind within the cleft of the large polymerase domain and adjacent

to the O helix. The results of these crystallographic studies were consistent with that of most biochemical and mutational investigations. However, at 3.9 Å resolution, the precise location and position of the phosphate groups could not be determined, neither could the precise orientation of the bases.

Here we present the high resolution crystal structures of binary complexes of Klentaq1 with all four deoxyribonucleoside triphosphates. The positions of the phosphate groups, as well as those of the sugar and the base, were determined unambiguously and shown to be consistent with mutational studies on Taq polymerase. Together with the structure by Eom et al. (1996), these structures provide a conceptual framework onto which an understanding of DNA replication by DNA Pol I enzymes can be built.

Results and discussion

Overall location and configuration of the dNTPs at the nucleotide binding site

The dNTP molecules bind to the cleft of the polymerase domain of Klentaq1, adjacent to the O helix (Figs. 1, 2A). The phosphate moiety of the nucleotides runs parallel to the O helix with the base moiety pointing toward the base of the cleft, i.e., toward the polymerase active site. This configuration of the nucleotides resembles that of the nucleotides seen in the binary complexes of *E. coli* Klenow Pol I (Beese et al., 1993). However, the much higher

resolution achieved here (2.4–2.5 Å) resulted in clear density for the base in all four binary complexes (Fig. 1) and the orientation of the nucleotides could be unambiguously obtained. Our results confirm that incoming dNTPs enter the cleft of the polymerase in a position favorable to insertion and reaction at the polymerase active site.

Interestingly, the co-crystal structure of Taq polymerase with duplex DNA at the polymerase active site shows that the blunt end of the duplex DNA is packed against the O helix (Eom et al., 1996). When the structures of the binary complexes of Klentaq1 with dATP and dCTP are superimposed onto the protein/DNA complex of Eom et al. (1996), the base of the free nucleotide is seen in a stacking arrangement with the 3' end nucleotide base of the primer strand (Fig. 2D), suggesting that the positions of dNTPs in these two binary complexes represent functional states of the polymerase reaction. In contrast, the base of the dTTP and dGTP nucleotides is seen in a different configuration (see detailed discussion in the paragraph below) such that the base would sterically collide with the 3' end of the primer strand: these complexes may not represent functional states of the reaction.

The positions of the triphosphate moiety of the nucleotides in the four Klentaq1/dNTP binary complexes are nearly identical, while the sugar and bases point in different directions (Fig. 2C). Such an observation suggests that the primary dNTP recognition event is that of the phosphate moiety. The base is seen in two different conformations. One conformation observed in the dATP-

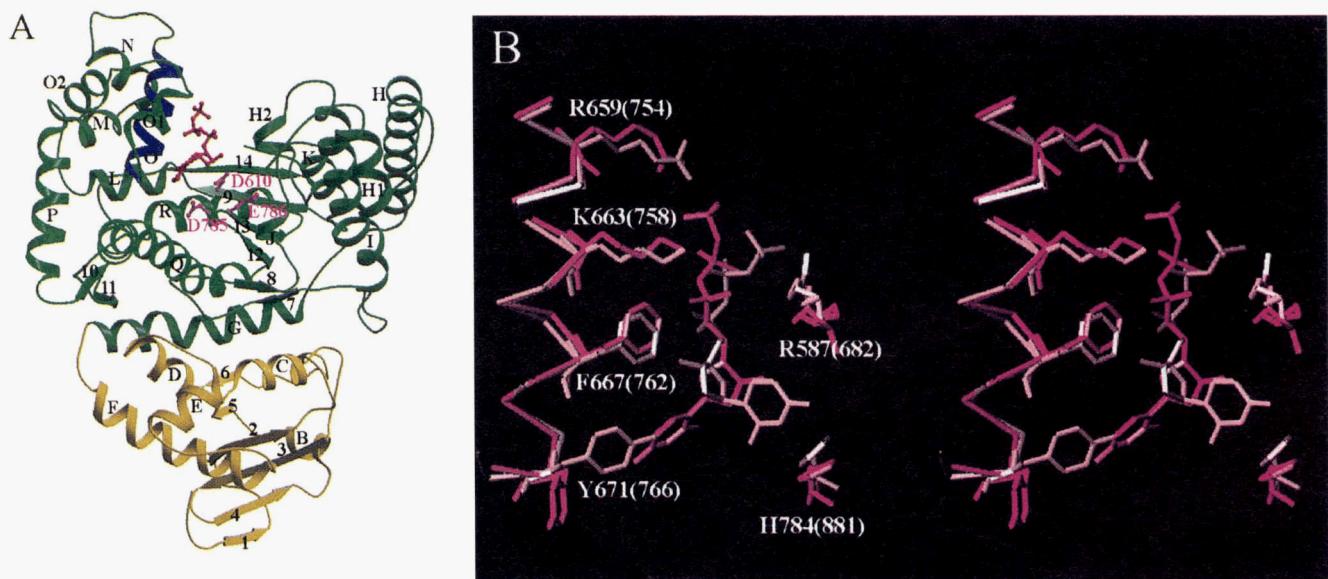


Fig. 2. **A:** Schematic representation of the secondary structure of Klentaq1 bound with dATP. The small vestigial 3'-5' exonuclease domain is shown in yellow and the polymerase domain is shown in green. The secondary structure elements are labeled according to the notation of Ollis et al. (1985). The helix O is shown in blue and the dATP molecule in red. The side chains of the three carboxylates, Asp610, Asp785, Asp786, which form the catalytic core are shown in purple. This figure was prepared using MOLSCRIPT and RASTER3D (Merritt & Murphy, 1994; Kraulis, 1991). **B:** Superimposition of the binary complex of Klentaq1 bound to dCTP (this work; in purple) with the binary complex of *E. coli* Klenow Pol I bound to dCTP ((Beese et al., 1993); in pink). **C:** Superimposition of dNTPs in the four Klentaq1/dNTP binary complexes. The binary complexes of Klentaq1 with dATP (green), dTTP (yellow), dCTP (magenta), dGTP (cyan) were superimposed. The positions of the triphosphates of the dNTP in the four complex are nearly identical, while those for the sugar and base differ. The orientation of the side chain of Tyr671 also differs in the four binary complexes. **D:** Superimposition of the Klentaq1/dCTP binary complex structure (in purple) with the Taq/DNA complex structure of Eom et al. (1996) (in yellow for the protein, and white and blue for the template/primer DNA strands, respectively). The dCTP base is seen in a stacking arrangement with the 3' end base of the primer strand, suggesting that this complex is functionally relevant. (Figure continues on facing page.)

and dCTP-Klentaq1 binary complexes is such that it is positioned to form a stacking arrangement with the primer strand of the DNA as observed by Eom et al. (1996). The other conformation observed in the dGTP- and dTTP-Klentaq1 complexes represents an approximately 90° and 120° rotation around the phosphate-ribose bond relative to the conformation of the base in the other complexes, respectively. Therefore, it appears that the base moieties in the nucleotides can adopt a wide set of configurations. However, only one of them (that seen in this report for dATP and dCTP) is compatible with the structure imposed by binding of DNA. Therefore, it would appear that the binding of DNA imposes structural constraints on the configuration of the base so that proper stacking arrangements between the 3' end base of the primer strand and the base of the incoming nucleotide can form.

The overall structure of dNTP-bound Klentaq1 remains the same as that of the apo form.

Interaction between the triphosphate group of dNTP and side chains of the protein

As observed in *E. coli* Pol I, the triphosphate group of the dNTP is within the range of electrostatic interaction with the positively charged side chains of Arg659 (754 in *E. coli* Pol I), Lys663 (758 in *E. coli* Pol I), Arg587 (682 in *E. coli* Pol I), and the side chains of His639 (734 in *E. coli* Pol I) and Gln613 (708 in *E. coli* Pol I) (Figs. 2, 3). The side chain of Arg659 is interacting with the γ phosphate, and that of Arg587 is interacting with the α phosphate. The side chain of Lys663 is within 4 Å of both the α and γ phosphate groups. The side chains of His639 and Gln613 are in the range of interaction with the γ and β phosphate groups, respectively (Fig. 3).

It has been shown by random mutagenesis study that the residues Arg659 and Lys663 in the O helix of Taq polymerase are

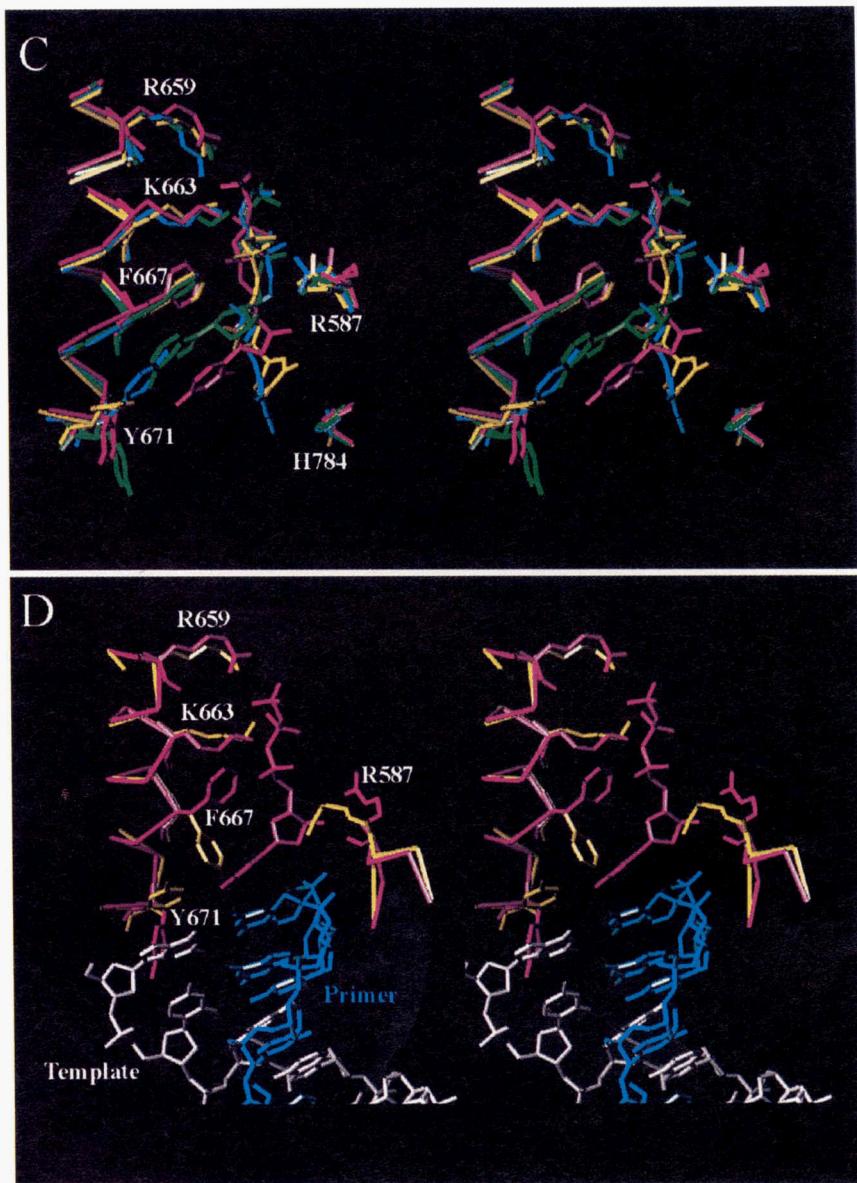


Fig. 2. *Continued.*

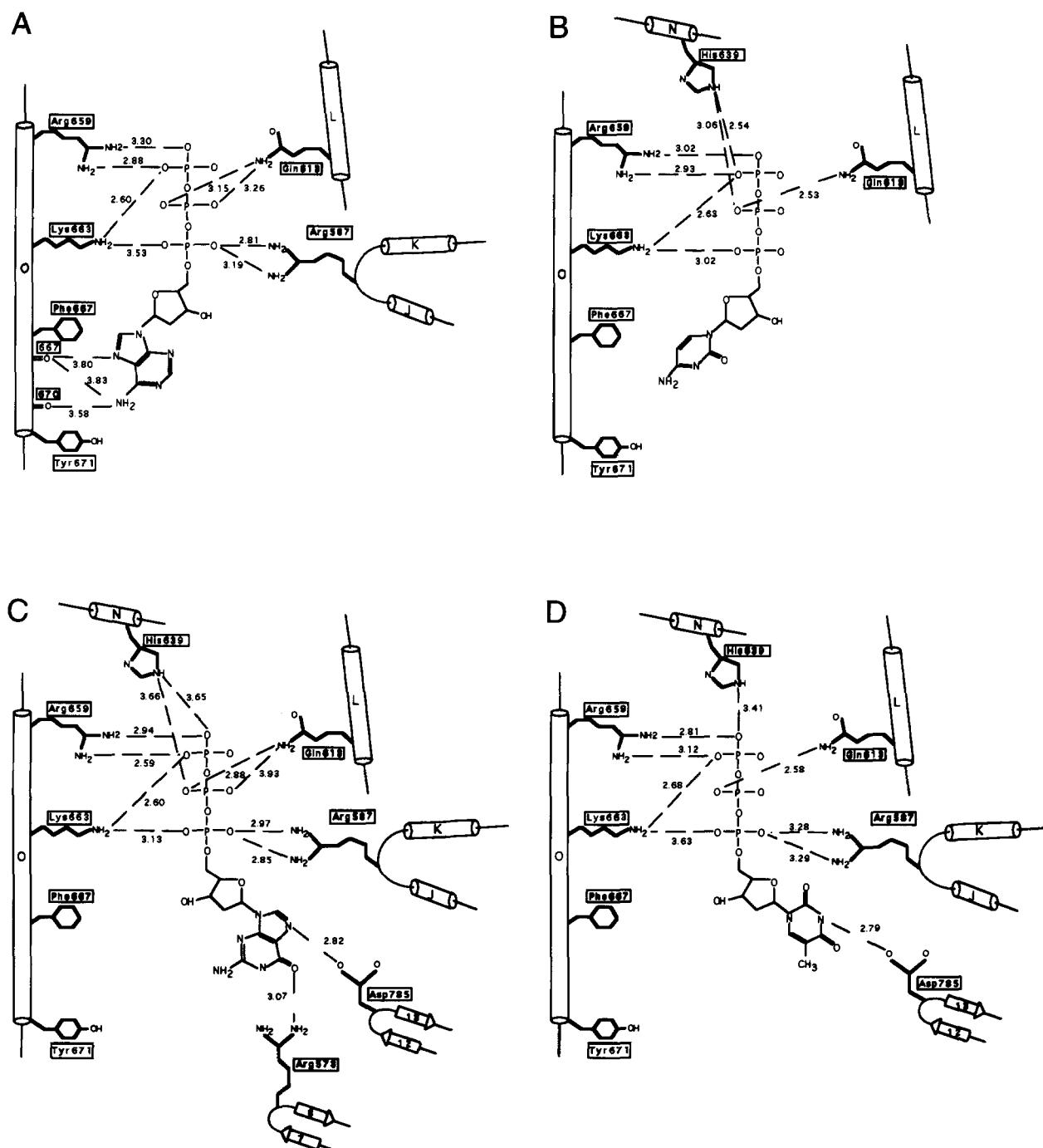


Fig. 3. Distances between the nucleotides and interacting protein side chains. Only potential H-bonds are shown. **A:** Klentaq1 with dATP. **B:** Klentaq1 with dCTP. **C:** Klentaq1 with dGTP; **D:** Klentaq1 with dTTP.

essential for function (Suzuki et al., 1996). Mutations of the corresponding residues (Arg754 or Lys758) in *E. coli* Pol I greatly affect the $K_m(\text{dNTP})$ and $K_m(\text{PP}_i)$ and have little or no effect on DNA binding (Astatke et al., 1995). These data suggest that Arg659 and Lys663 or equivalents are integral parts of the nucleotide binding site of DNA Pol I enzymes. The binary complex structures presented here bring additional evidence for the functional importance of these residues: the side chains of Arg659 and Lys663 both interact with the γ phosphate group of the incoming substrate

dNTP, while Lys663 provides additional contacts with the α phosphate group. Also consistent with biochemical evidence is the observation by Eom et al. (1996) that Arg659 and Lys663 are not interacting with DNA (Eom et al., 1996).

The corresponding residue of Arg587 in Klentaq1, Arg682 in *E. coli* Pol I, was found to be the site of 5'-fluorosulfonyladenosine (5'-FSBA) crossing-linking (Pandey & Modak, 1988). 5'-FSBA is a structural analogue of ATP, with its active sulfonyl fluoride moiety thought to occupy a position similar to that of the β and γ

phosphates of ATP (Wyatt & Colman, 1977). The crystal structure of the binary complex of Klenow *E. coli* Pol I with dCTP (Beese et al., 1993) shows that the side chain of Arg682 is indeed within H-bond distance of the β and γ phosphate groups of the nucleotide, thereby providing a structural basis for the results of the cross-linking experiments by Pandey and Modak (1988). However, in the binary complex structures of Klentaq1 and dNTP (this report), Arg587 is seen interacting with the α -phosphate, and not with the β and γ phosphates. These interactions result from a different positioning of the β and γ phosphates relative to the positions of these phosphates in the Klenow Pol I-dCTP complex structure (Fig. 2B). Our results seem to conflict with the biochemical evidence provided by Pandey and Modak (1988). However, a subsequent study by the same authors and others (Pandey et al., 1993; Astatke et al., 1995) reveal a more complex role for Arg682 (587 in Klentaq1): mutations at this site do not abrogate polymerase activity and kinetic studies on the protein containing a R682A mutation shows only a small increase in $K_m(\text{dNTP})$ (Pandey et al., 1993; Astatke et al., 1995). These results were interpreted as indicative of a possible involvement of Arg682 in DNA binding (Pandey et al., 1993; Astatke et al., 1995). Indeed, Arg587, the amino acid equivalent to Arg682 in *E. coli* Pol I, is seen in the co-crystal Taq/DNA structure of Eom et al. (1996) within electrostatic interaction of the DNA phosphate backbone. Also consistent with this interpretation is that the side chain conformation of Arg587 in the binary complex structures presented here is more similar to that of the same residue in the DNA-Taq complex structure than that of the equivalent residue (Arg682) in the binary Klenow Pol I-dNTP complex structure (Fig. 2B,D): this suggests that the conformation seen here (i.e., a side chain interacting with the α -phosphate rather than the more distal phosphate groups) reflects a functional state of this side chain conformation. A second possible explanation for the discrepancy between the structures presented here and that of Beese et al. (1993) is that the side chain of Arg587 (682 in *E. coli*) does not make specific interactions with dNTP and that the positioning of the nucleotides in the binary complexes is dependent on the solution conditions used for crystallization.

Residues His639 and Gln613 are highly conserved among the DNA Pol I family of proteins, suggesting that these residues may play a role in the function of polymerases. Our crystal structure is consistent with that of the binary complex of Klenow *E. coli* Pol I with dCTP (Beese et al., 1993) in that the side chains of His639 (734 in *E. coli* Pol I) and Gln613 (708 in *E. coli* Pol I) are close enough to the β and γ phosphates of dNTP to make direct contact (Figs. 2, 3). However, mutations H734A or Q708A in *E. coli* Pol I cause only small or modest effects on $K_m(\text{dNTP})$ (Astatke et al., 1995), suggesting that these residues may not interact with dNTP directly in the active ternary complex (Astatke et al., 1995). Astatke et al. (1995) proposed that His639 and Gln613 may interact with single-stranded DNA template beyond the site of nucleotide addition (Astatke et al., 1995). As suggested by Astatke et al. (1995), formation of an active ternary complex of the polymerase with both template-primer DNA and substrate dNTP may be required to position part of the DNA near His639 and Gln613. The answer to this question may await the determination of the co-crystal structure of such an active ternary complex.

Structural basis for the function of Phe667

Phe667 is a highly conserved residue on the O helix. Random mutagenesis study shows that this residue is important for function

(Suzuki et al., 1996). The side chain of Phe667 is interacting with the base of the primer strand at the blunt end of the duplex DNA, as shown in the co-crystal structure of Taq polymerase with duplex DNA at the polymerase active site (Eom et al., 1996). In our binary complex of Klentaq1 with dNTP, the phenyl ring of Phe667 forms a platform which runs mostly parallel to the sugar rings of the incoming dNTPs. This side chain arrangement is consistent with findings by Astatke et al. (1995), which have shown that a substitution to alanine at the equivalent position (residue Phe762) in *E. coli* pol I causes a large increase in $K_m(\text{dNTP})$ while causing almost no effect on $K_m(\text{PP}_i)$ (Astatke et al., 1995), indicating that Phe762 in *E. coli* Pol I, or the equivalent Phe667 in Taq polymerase, does not interact with the nucleotide's phosphate moiety.

It has been shown that the side chain of Phe667 is critical in distinguishing dNTPs from ddNTPs as substrates (Tabor & Richardson, 1995). When Phe667 is mutated to Tyr, the ability of the polymerase to distinguish ddNTPs drops 250- to 8,000-fold (Tabor & Richardson, 1995). A mechanism for the discrimination has been proposed that suggests that one hydroxyl group may be required, either from the nucleotide or from the enzyme, for binding of a metal ion critical for the DNA polymerizing reaction. This would imply that the 3'-hydroxyl group of the deoxyribose, at some stage in the DNA polymerizing reaction, is close enough to the ζ -carbon of the phenyl ring. Unfortunately, in our binary crystal structures, the ζ -carbon of the side chain of Phe667 is not seen anywhere near the 3' ribose hydroxyl and therefore, the structure of binary complexes cannot provide a structural basis for the remarkable properties of the F667Y mutation. However, some possible explanation for the effect of this mutation may come from the structural superimposition of the Klentaq1/dNTP binary complexes and the Taq/DNA complex of Eom et al. (1996) (Fig. 2D). It then becomes apparent that the conformation of the side chain of Phe667 in the binary complex is very different from that seen in the protein/DNA complex (Fig. 2D): while the side chain is orientated outwards (away from the catalytic core) in the binary dNTP complexes, it is orientated inward (toward the polymerase catalytic core) in the DNA complex. It is therefore possible that during binding and catalysis, the side chain undergoes a conformational change resembling a lever, that accompanies entry of the incoming nucleotide. At some stage during that conformational change, the 3' ribose hydroxyl may come close to the tip of Phe667, a mechanism which would then account for the effect of F667Y.

The side chain conformation of Tyr671 and the orientation of the base

The conformations of the side chain of Tyr671 in the four binary complexes differ greatly and no contact is observed between this side chain and the nucleotide. However, electron density for the side chain was weak, indicating conformational flexibility. There is indirect evidence that the side chain of Tyr671 interacts with the incoming nucleotide (Joyce et al., 1986). However, the most convincing evidence suggests that Tyr671 (Tyr766 in *E. coli*) interacts with DNA (Catalano et al., 1990; Astatke et al., 1995; Eom et al., 1996). Interestingly, the phenyl ring of Tyr671 in the Taq/DNA complex of Eom et al. (1996) is seen forming a stacking interaction with the 5' template end base. On the other hand, in the superimposition of the structure of the Taq-DNA complex with that of the dATP- and dCTP-Klentaq1 binary complexes presented here (Fig. 2D), the base of the incoming nucleotide forms a stacking

interaction with the 3' end of the primer strand, and the ring of the incoming base and that of Tyr671 are co-planar. This suggests that the side chain of Tyr671 may play a crucial role in orienting the base of the incoming nucleotide for proper insertion into the DNA chain.

Residues of the catalytic core

Residues Asp785, Glu786, and Asp610 (882, 883, and 705 in *E. coli*) are known to form the catalytic core of the polymerase domain and Asp785 is seen within H-bond distance interaction of the base in the dGTP and dTTP-Klentaq1 binary complexes (Fig. 3C,D). However, such an interaction is not seen in the dATP- and dCTP- Klentaq1 binary complexes (Fig. 3A,B). The three carboxylates function presumably by binding to metal ions which would promote the deprotonation of the 3' OH of the primer strand and/or facilitate the formation of the pentacovalent transition state at the α -phosphate of the dNTP and the release of pyrophosphate (Joyce & Steitz, 1994). Therefore it is unlikely that the interactions of the base of the incoming nucleotide with Asp785 as observed in the binary complexes of Klentaq1 with dGTP or dTTP reported here correspond to a functional intermediate of the polymerase reaction.

Conclusion

The crystal structures of binary complexes of Klentaq1 with dNTPs presented here represent additional structural information which contribute to understanding the function of the DNA polymerase I enzymes. The high resolution achieved for these structures yields a precise definition of the structures of the dNTPs and their posi-

tioning relative to the protein. At least two of the dNTPs (dATP and dCTP) are seen in a conformation in which stacking of the base onto the DNA could occur. These structures also provide a basis for the role of some of the side chains at or near the dNTP binding site. However, the answers to some of the most pressing questions, such as those relating to the issue of the structural arrangements of the various binding partners during DNA polymerization, will need to await the determination of active ternary complex structures of the protein with template-primer DNAs and dNTPs.

Materials and methods

Crystals of uncomplexed Klentaq1 were grown as described in Korolev et al. (1995) and harvested into a stabilizing solution containing 12% (wt/vol) polyethylene glycol 3350, 50 mM MgCl₂, and 100 mM Tris HCl, pH 9.0. The crystals were then transferred into a solution containing 12% (wt/vol) polyethylene glycol 3350, 50 mM MgCl₂, and 100 mM Tris HCl, pH 9.0, and 5 mM of one of the four nucleotides triphosphates (dATP, dTTP, dGTP, and dCTP) typically for 24 h. Diffraction data for the dTTP, dGTP, and dCTP-Klentaq1 complexes were collected at room temperature using a Rigaku RaxisII image plate detector mounted on a Rigaku RU200 copper rotating anode. Oscillation angles were typically 2°. Data were processed and scaled using the programs DENZO and SCALEPACK (Otwinowski, 1993) (Table 1). For the dATP-Klentaq1 complex, data were collected at room temperature using a Hamlin multiwire area detector (Hamlin, 1985) mounted on a Rigaku RU200 copper rotating anode. Data processing and scaling proceeded using the package provided by the manufacturer (Howard et al., 1985).

Table 1. Summary of crystallographic data and refinement

| | Taq/dATP | Taq/dGTP | Taq/dTTP | Taq/dCTP |
|---|----------------|---------------|---------------|---------------|
| Crystals | | | | |
| Resolution (Å) | 2.4 | 2.4 | 2.5 | 2.5 |
| Reflections (observed/unique) | 10,9042/27,872 | 60,651/23,975 | 66,484/21,846 | 62,452/21,040 |
| Completeness ^a (%), all data | 99.6/99.8 | 86.3/69.8 | 88.3/78.9 | 85.9/74.8 |
| R_{sym} ^b (%) | 6.5 | 8.0 | 8.0 | 6.9 |
| Refinement | | | | |
| R factor (%) | 22.3 | 22.4 | 22.4 | 21.7 |
| R_{free} ^c (%) | 26.5 | 27.5 | 29.3 | 28.0 |
| Resolution | 6.0–2.4 | 6.0–2.4 | 6.0–2.5 | 6.0–2.5 |
| Completeness ^a (%), $I/\sigma > 2$ | 90.5/86.5 | 81.2/64.5 | 83.5/72.6 | 80.8/65.6 |
| Total no. of atoms | 5,214 | 5,216 | 5,212 | 5,212 |
| Average B factor for main chain atoms | 23.7 | 30.4 | 31.4 | 29.1 |
| Average B factor for side chain atoms | 25.4 | 29.9 | 30.4 | 29.0 |
| RMS deviation in bond length (Å) | 0.014 | 0.014 | 0.014 | 0.014 |
| RMS deviation in bond angle (°) | 3.203 | 3.142 | 3.277 | 3.170 |
| RMS deviation in B for bonded atoms (Å ²) | | | | |
| Mainchain | 0.847 | 0.839 | 0.855 | 0.849 |
| Sidechain | 3.203 | 3.772 | 3.873 | 3.447 |

^aPercentage of overall completeness and completeness in the last resolution shell (2.40–2.44 Å for Klentaq1/dATP and Klentaq1/dGTP and 2.50–2.54 Å for Klentaq1/dTTP and Klentaq1/dCTP), respectively. RMS bond lengths and angles are the deviations from ideal values.

^b $R_{sym} = \sum |I - \langle I \rangle| / \sum I$, where I = observed intensity, $\langle I \rangle$ = average intensity from multiple observations of symmetry related reflections.

^cFree R -values were calculated using 5% of the data set.

Soaks of dNTPs into apo Klentaq1 crystals did not result in any substantial degradation in crystal quality. Only a weak drop in resolution was observed (2.5 Å against 2.4 Å for the apo Klentaq1) for crystals soaked in dTTP and dCTP. The space group (P21212) and cell dimensions ($a = 109.4$ Å, $b = 136.8$ Å, $c = 45.6$ Å) of the binary complexes were nearly identical to that of apo Klentaq1. Therefore, the structure of apo Klentaq1 was directly submitted to positional refinement procedures against the binary complex data (program XPLOR (Brünger, 1992)). An $F_o - F_c$ map was then calculated using the coordinates of the resulting refined apo structure for each binary complex and resulted in clear electron density for both the phosphate and base regions in the four nucleotides (Fig. 1). The dNTP molecules were fitted into the electron density [program O (Jones & Thirup, 1986; Jones et al., 1991)] and subjected to positional refinement and simulated annealing procedures followed by B -factor refinement (Brünger, 1992). Mg ions were not incorporated into the model. B -factors for the nucleotides are similar in values to those of the surrounding protein atoms. The summary of data analysis and the refinement is shown in Table 1.

Structural comparison with the *E. coli* Klenow fragment-dCTP binary complex structure (Beese et al., 1993) and with the Taq polymerase-blunt end DNA duplex complex structure (Eom et al., 1996) was carried out using coordinates deposited at the Brookhaven Data Bank with accession codes 1KFD (Klenow fragment-dCTP binary complex) and 1TAU (Taq polymerase-blunt end DNA duplex complex).

Acknowledgments

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References

- Astatke M, Grindley NDF, Joyce CM. 1995. Deoxynucleoside triphosphate and pyrophosphate binding sites in the catalytically competent ternary complex for the polymerase reaction catalyzed by DNA polymerase I (Klenow fragment). *J Biol Chem* 270:1945–1954.
- Barnes WM. 1992. The fidelity of Taq polymerase catalyzing PCR is improved by an N-terminal deletion. *Gene* 112:29–35.
- Barnes WM. 1994. PCR amplification of up to 35 kb DNA with high fidelity and high yield from λ templates. *Proc Natl Acad Sci USA* 91:2216–2220.
- Beese LS, Friedman JM, Steitz TA. 1993. Crystal structure of the klenow fragment of DNA polymerase I complexed with the deoxynucleoside triphosphate and pyrophosphate. *Biochemistry* 32:14095–14101.
- Brünger A. 1992. *X-PLOR (Version 3.1) Manual*. New Haven: Yale University.
- Catalano CE, Allen DJ, Benkovic SJ. 1990. Interaction of *Escherichia coli* DNA polymerase I with azidoDNA and fluorescent probes: Identification of protein-DNA contacts. *Biochemistry* 29:3612–3621.
- Cheng S, Fockler C, Barnes WM, Higuchi, R. 1994. Effective amplification of long targets from cloned inserts and human genomic DNA. *Proc Natl Acad Sci USA* 91:5695–5699.
- Delarue M, Poch O, Tordo N, Moras D, Argos P. 1990. An attempt to unify the structure of polymerases. *Protein Eng* 3:461–467.
- Eom SH, Wang J, Steitz TA. 1996. Structure of Taq polymerase with DNA at the polymerase active site. *Nature* 382:278–281.
- Hamlin R. 1985. Multiwire area X-ray diffractometer. *Methods Enzymol* 114:416–452.
- Howard AJ, Nielsen C, Xuong NH. 1985. Software for a diffractometer with multiwire area detector. *Methods Enzymol* 114:452–472.
- Jones TA, Thirup S. 1986. Using known substructures in protein model building and crystallography. *EMBO J* 5:819–822.
- Jones TA, Zou JY, Cowan SW, Kjeldgaard M. 1991. Improved methods for building protein models in electron density maps and the location of errors in these models. *Acta Crystallogr A47:110–119*.
- Joyce CM, Ollis DL, Rush J, Steitz TA, Konigsberg WH, Grindley NDF. 1986. Relating structure to function for DNA polymerase I of *Escherichia coli*. In: *Protein structure, folding, and design*. New York: Alan R. Liss. pp 197–205.
- Joyce CM, Steitz TA. 1994. Function and structure relationships in DNA polymerases. *Ann Rev Biochem* 63:777–822.
- Kim Y, Eom SH, Wang J, Lee D-S, Suh SW, Steitz TA. 1995. Crystal structure of *Thermus aquaticus* DNA polymerase. *Nature* 376:612–616.
- Kornberg A, Baker TA. 1991. *DNA replication*. 2nd ed. New York: W.H. Freeman.
- Korolev S, Nayal M, Barnes W, Di Cera E, Waksman G. 1995. Crystal structure of the large fragment of *Thermus aquaticus* DNA polymerase I at 2.5 Å resolution: Structural basis for thermostability. *Proc Natl Acad Sci USA* 92:9264–9268.
- Kraulis PJ. 1991. MOLSCRIPT: A program to reproduce both detailed and schematic plots of protein structure. *J Appl Crystallogr* 24:946–950.
- Lawyer FC, Stoffel S, Saiki RK, Chang S-Y, Landre PA, Abramson RD, Gelfand DH. 1993. High-level expression, purification, and enzymatic characterization of full-length *Thermus aquaticus* DNA polymerase and a truncated form deficient in 5' to 3' exonuclease activity. *PCR Methods and Applications* 2:275–287.
- Merritt EA, Murphy MEP. 1994. Raster3D Version 2.0: A program for photo-realistic molecular graphics. *Acta Crystallogr D50:869–873*.
- Ollis DL, Brick P, Hamlin R, Xuong NG, Steitz TA. 1985. Structure of a large fragment of *Escherichia coli* DNA polymerase I complexed with dTMP. *Nature* 313:762–766.
- Otwinowski Z. 1993. In: Sawyers L, Isaacs N, Bailey S, eds. *Proceedings of the CCP4 study weekend: Data collection and processing*. Warrington: SERC Daresbury Laboratory. pp 56–62.
- Pandey VN, Kaushik N, Sanzgiri RP, Patil MS, Modak MJ, Barik S. 1993. Site directed mutagenesis of DNA polymerase I (Klenow) from *Escherichia coli*. The significance of Arg682 in catalysis. *Eur J Biochem* 214:59–65.
- Pandey VN, Modak MJ. 1988. Affinity labeling of *Escherichia coli* DNA polymerase I by 5'-fluorosulfonylbenzoyladenosine. Identification of the domain essential for polymerization and arg-682 as the site of reactivity. *J Biol Chem* 263:6068–6073.
- Suzuki M, Baskin D, Hood L, Loeb LA. 1996. Random mutagenesis of *Thermus aquaticus* DNA polymerase I: Concordance of immutable sites *in vivo* with the crystal structure. *Proc Natl Acad Sci USA* 93:9670–9675.
- Tabor S, Richardson CC. 1995. A single residue in DNA polymerase of the *Escherichia coli* DNA polymerase I family is critical for distinguishing between deoxy- and dideoxyribonucleotides. *Proc Natl Acad Sci USA* 92:6339–6343.
- Wyatt JL, Colman RF. 1977. Affinity labeling of rabbit muscle pyruvate kinase by 5'-p-fluorosulfonylbenzoyladenosine. *Biochemistry* 16:1333–1342.