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Chapter XIII

Taq DNA Polymerase Interactions with DNA and Fab

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Abstract

The *Taq* DNA polymerase from *Thermus aquaticus* is the most commonly used enzyme for *in vitro* DNA amplification using polymerase chain reaction (PCR). This enzyme consists of the polymerase (homologous to *Escherichia coli* DNA polymerase 1), the nuclease and the vestigial (intervening) editing domains. The structures of *Taq* DNA polymerase in complex form with DNA and Fab (antibody fragment) is now available at the protein databank (PDB). Therefore, it is important to document the common structural features essential for DNA and protein binding with *Taq* DNA polymerase. Hence, we created a dataset consisting of twelve (12) *Taq* DNA polymerase structures in complex with DNA and/or Fab (fragment of antibody). It is our interest to document the physical-chemical characteristics of the interfaces between *Taq* polymerase with DNA and/or Fab. The DNA binding region is predominantly hydrophilic in nature for DNA (> 70%) and Fab binding (~ 68%). The Fab binds to Taq polymerase such that the activity of *Taq* is transiently inhibited for hot-start PCR (for improved specificity). Nonetheless, the Taq-Fab interface is also prevailingly hydrophilic (~68%). These observations have application in the design and development of better *Taq* DNA polymerase for effective PCR. Our observation suggests specific yet sensitive nature of protein-protein interactions in the case of *Taq* polymerase with Fab molecule.

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13.1. Introduction

The DNA polymerase from *Thermus aquaticus (Taq* polymerase) belongs to the family of DNA polymerase 1 enzymes which play a role in the repair of DNA lesions in prokaryotic organisms (Delarue *et al*. 1990). *Taq* polymerase is a single monomeric enzyme with a molecular weight of 93.9 kDa (Lawyer *et al*. 1988). *Taq* polymerase consists of three domains like the other members of the polymerase 1 family (Figure 13.1 and 13.2). The enzyme consists of an N-terminal domain (residues 1-290) having a 5'-3' nuclease activity, an intervening domain (residues 291-419), and a C-terminal polymerase domain (residues 420-832). The polymerase domain is homologous to the large domain of the klenow fragment in *E.coli* Polymerase 1(Kim *et al.* 1995).The structure of native *Taq* polymerase shows three metal ions (two Mn^{2+} and one $Zn^{2+,}$) at the active site of the nuclease domain. This is in agreement with the two metal ion mechanism proposed for nuclease activity (Beese *et al*. 1991). The *Taq* polymerase structures have been solved in two different orientations (nuclease domain in elongated (Kim *et al.* 1995) and compact conformations (Urs *et al.* 1999). Allison *et al.* (2003) studied the global conformation and hydro-dynamics of full length *Taq* and full length *E. coli* polymerase 1. They showed that they exist in the elongated conformation in the absence of a bound DNA.

The *Taq* DNA polymerase was earlier described as the right hand in which the palm, fingers and thumb form the DNA-binding crevice (Ollis *et al.* 1985). Eom *et al.* (1996) solved the polymerase structure with the blunt-ended duplex DNA (GCGATCCG) (Figure 13.3). Murali *et al.* (1998) showed the interaction of inhibitory Fab in *Taq* polymerase activity. They solved the TP7 antibody (for polymerase inhibition at room temperature) bound to the native enzyme as applied in hot-start PCR. Li and co-workers (1998) provided the crystal structures of open and closed forms (different orientation of fingers domain) of binary and ternary complexes of the large fragment of *Taq* DNA polymerase 1. The structures show that O-helix conformational change lead to the reorientation of finger domains. Li *et al.* (1999) have determined the crystal structure of all four ddNTP-trapped closed ternary complexes of the *Taq* polymerase. They showed that ddGTP-trapped complex (as R660 formed hydrogen bond with the ddGTP base) is different from other three complexes (ddATP, ddTTP, and ddCTP). Hence, mutants of R660 (R660D, R660L, R660S, R660Y, R660F) was developed to enhance the activity of the *Taq* polymerase.

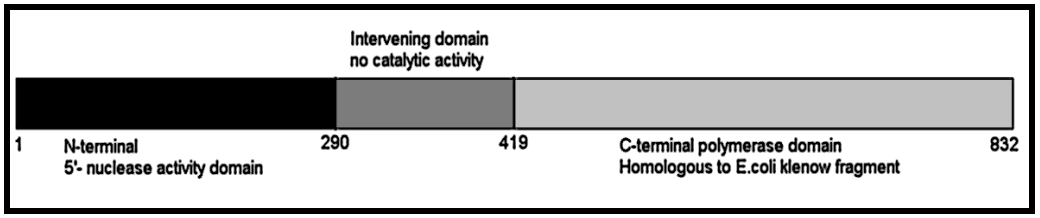

Figure 13.1. One dimensional diagram of *Taq* DNA polymerase. The *Taq* DNA polymerase consists of three domains. They are nuclease domain (1-290 residues – red color), intervening domain (291-419 residues – yellow color) and polymerase domain (420-832 residues – blue color). It should be noted that C-terminal polymerase domain is homologous to the *E. coli* polymerase domain.

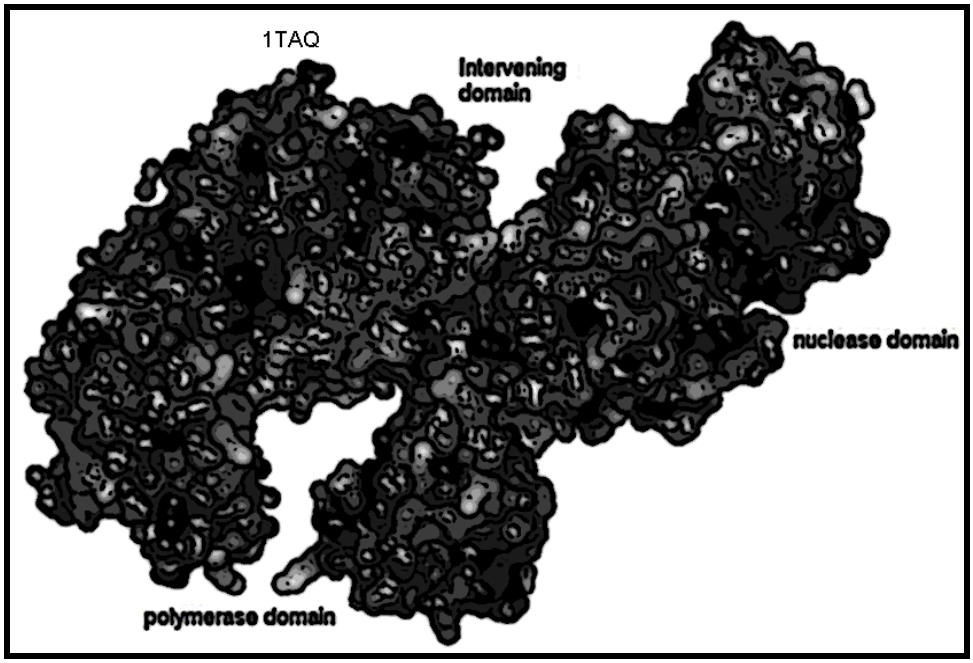

Figure 13.2. The 3D structure of the *Taq* DNA polymerase (PDB ID: 1TAQ) has been known since 1995 (Kim *et al*. 1995; Korolev *et al*. 1995). Like *E. coli* pol 1; *Taq* polymerase has a 5' nuclease domain at its N-terminus and a C-terminal polymerase domain. *E. coli* pol 1 contains a 3'-5' exonuclease activity in the intervening domain, whereas the corresponding domain in the *Taq* polymerase has lost this proofreading function. A comparison of the 3D structure of *Taq* polymerase with the klenow fragment structure showed that the polymerase domains are very similar, whereas the intervening domains differ broadly.

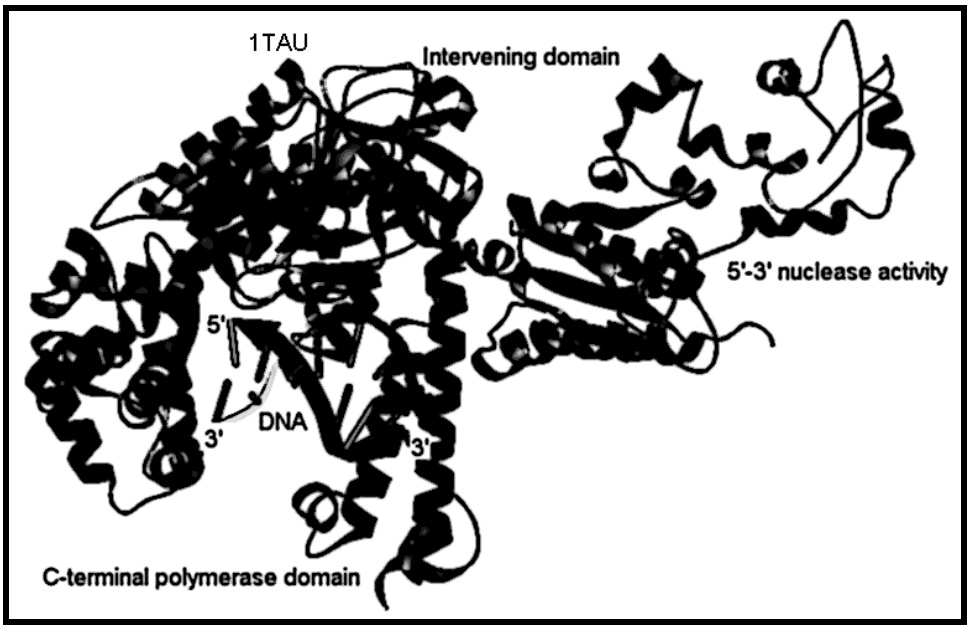

Figure 13.3. *Taq* DNA polymerase structure with the blunt-ended duplex DNA (GCGATCCG) (PDB ID: 1TAU). The DNA sequences used form 8 base pairs of duplex. Duplex DNA in this complex has both A and B form characteristics.

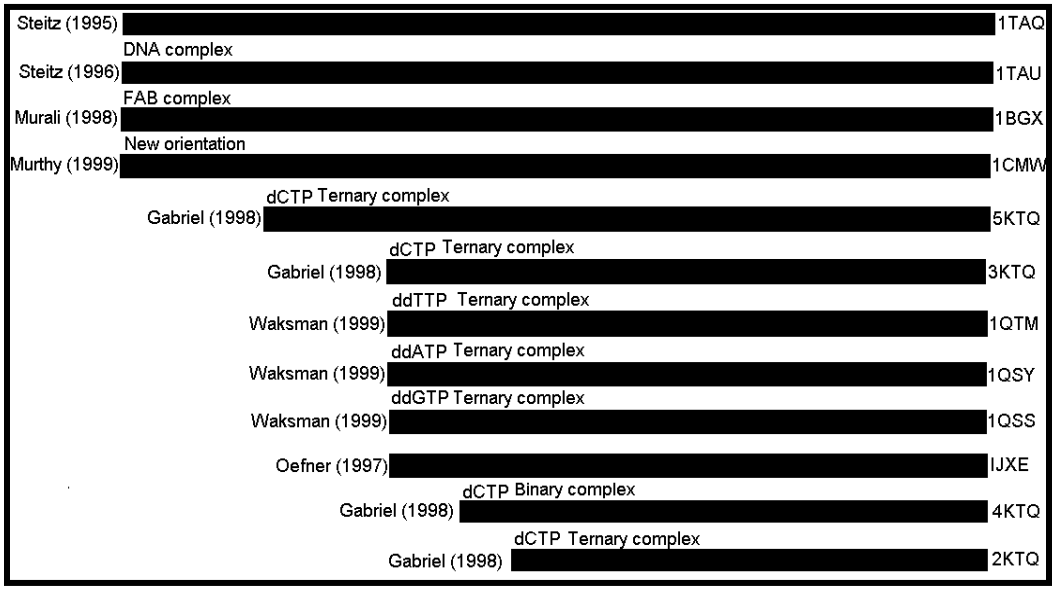

Figure 13.4. This is block diagram of the *Taq* DNA polymerase structures (12). This is created by performed the MSA sequences in the dataset. This is conducted by using clustalW2 program. The importance of the polymerase domain in DNA amplification is widely known and hence, this domain is highly represented in the dataset.

The major distinguishing feature of *Taq* polymerase is its extreme thermal stability. The enzyme can withstand temperatures in excess of 95°C for prolonged periods with an optimum of 75°C for reactions. It is also known that the rate of reaction is reduced to 50% at 60°C and to 10% at 37°C (Chien *et al.* 1976). *Taq* polymerase is rapidly replacing other DNA polymerase in many laboratory techniques, primarily because of its thermal stability and tolerance of temperature changes. This feature allows reaction to be performed at elevated temperature, whereby DNA duplex melting and annealing can be rigorously controlled.

The polymerase chain reaction (PCR) described elsewhere (Saiki *et al.* 1985) did not achieve its full potential until *Taq* DNA polymerase was adopted as the PCR synthetic enzyme. The PCR has applications beyond the research laboratory, including uses in forensic science, disease diagnosis or prognosis, paternity testing and animal and plant breeding programs (Ehrlich *et al.* 1991). The use of *Taq* polymerase in DNA sequencing with increased stringency of primer usage is also known (Ho *et al.* 2004). Thus, *Taq* polymerase is an important tool for the molecular biology experiments. Hence, it is important to understand molecular significance of *Taq* polymerase interactions with DNA and other proteins like Fab. This will help in the design of better *Taq* polymerase enzymes with increased catalytic efficiency. Here, we use structures of *Taq* polymerase enzymes in complex with DNA and Fab to glean structural features for potential design and development.

13.2. *Taq* DNA Polymerase Complex Dataset

We created a structural dataset of *Taq* DNA polymerase from Protein databank (PDB - http://www.rcsb.org/pdb/) (Table 13.1). The dataset consists of native *Taq* polymerase and its

complex with Fab and/or DNA. The structure of *Taq* polymerase (832 residues) consists of the nuclease (1-290), the vestigial (intervening) editing (291-419) and the polymerase domains (420-832) (Figure 13.1).

13.3. Multiple Sequence Alignment (MSA) of *Taq* DNA Polymerase Complex

MSA of sequences in the dataset was performed using the software tool clustalW2 (Thomson *et al.* 1994). The MSA was then used to create block diagrams of the structures as shown in Figure 13.4. The importance of the polymerase domain in DNA amplification is widely known and hence, this domain is highly represented in the dataset.

13.4. Accessible Surface Area (ASA) in *Taq* Polymerase Complexes

ASA of *Taq* polymerase and their complexes were calculated using the algorithm implemented by Lee and Richard (1971) in the software tool SURFACE RACER (Tsodikove *et al.* 2002). A probe radius of 1.4 Å was used in the calculations.

13.5. Interface Area in *Taq* Polymerase Complexes

Interface area between *Taq* polymerase and its complex state molecule is defined as the change in accessible surface area (ΔASA) from native enzyme state to a complex state. The involvement of *Taq* polymerase in complex formation is represented in a 2D graph as a function of residue function as shown in Figure 13.5 and 13.6.

13.6. Interface Property in *Taq* Polymerase Complexes

The binding of *Taq* polymerase with DNA or Fab is dependent on the interface interaction between them. This is usually deterministic of the physical-chemical properties of the interface. We calculated the prevalence of hydrophilic residues at the interface using the standard property based amino-acid residues classification system (Table 13.2).

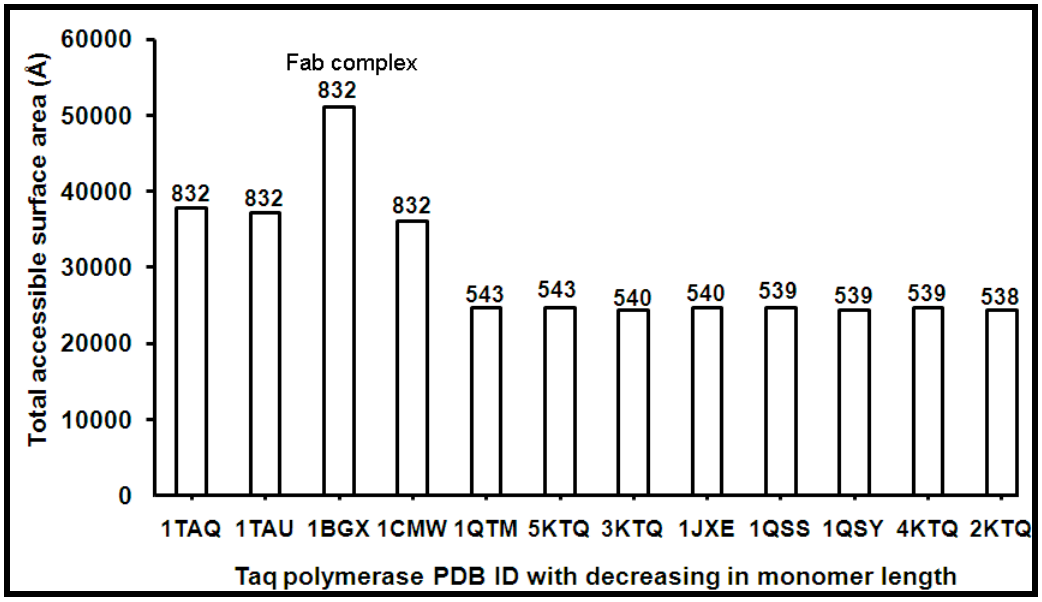

Figure 13.5. The total accessible surface area respective to the monomer length is shown here. The Fab complex (1BGX) has larger accessible surface area (~ 50000Å). 1TAQ, 1TAU and 1CMW structures have moderate accessible surface area (30000-40000Å). Structures namely 1QTM, 5KTQ, 3KTQ, 1JXE, 1QSS, 1QSY, 4KTQ and 2KTQ have small accessible surface area (20000-30000).

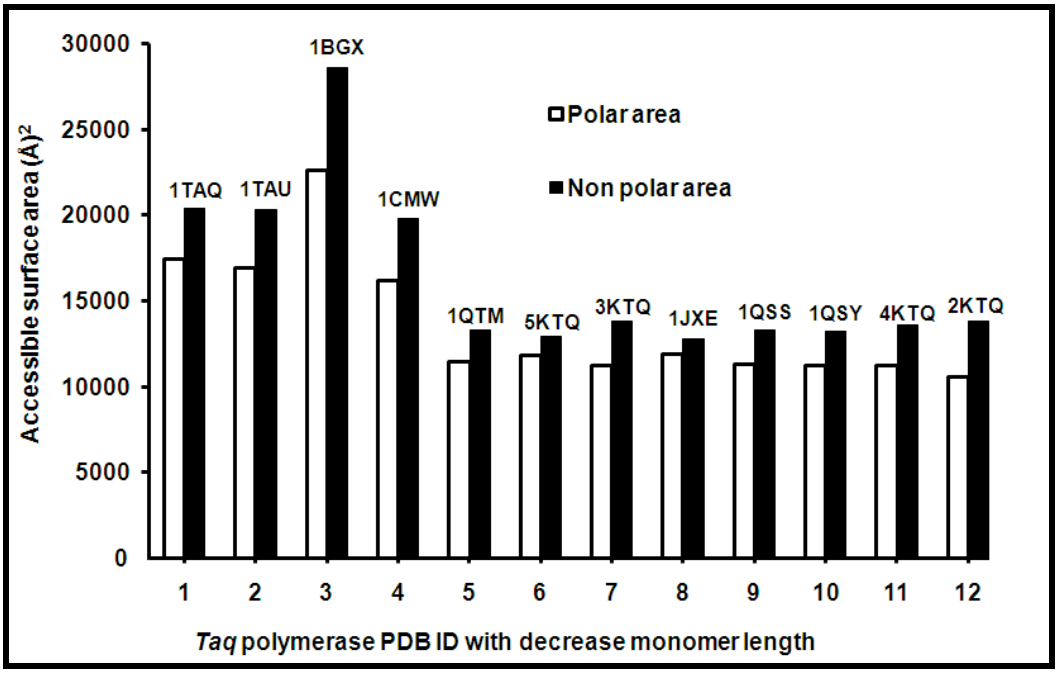

Figure 13.6. The polar and non-polar area of *Taq* DNA polymerase in the difference structure is shown in the above diagram. This shows that the major portion of the *Taq* DNA polymerase protein is associated with non-polar area while a small fraction of the protein is associated with polar area.

Table 13.2. Taq DNA polymerase complex interface properties

PDB ID	residues	interface residues	Hydrophilic (%)			Hydrophobic (%)		
			Total	Charged	Uncharged	Total	Aliphatic	Aromatic
1 TAU	832	49	75.51	30.61	44.90	24.52	18.40	6.12
1 BGX	832	91	68.14	34.07	34.07	31.87	23.08	8.79
1 QSS	539	69	72.45	31.88	40.57	27.54	21.74	5.80
1 QSY	539	65	73.85	32.31	41.54	26.16	21.54	4.62
1 QTM	543	68	70.59	30.88	39.71	29.41	23.53	5.88
2 KTQ	538	61	77.05	34.43	42.62	22.95	19.67	3.28
3 KTQ	540	69	73.91	31.88	42.03	26.09	20.29	5.80
4 KTQ	539	58	79.31	34.48	44.83	20.68	17.24	3.44
Average	612.75	66.25	73.85	32.57	41.28	26.15	20.69	5.47

1TAU (with DNA -GCGATCCG); 1BGX (with Fab); 1QSS (ddGTP-trapped complex); 1QSY (ddATP-trapped complex); 1QTM (ddTTP-trapped complex); 2KTQ (Open form ternary complex); 3KTQ (Closed form ternary complex); 4KTQ (Open form binary complex).

13.7. Interface Features in *Taq* Polymerase Complexes

The interaction between *Taq* DNA polymerase with Fab and DNA is of interest. The interface properties between *Taq* DNA polymerase and Fab are documented. Figure 13.5 gives the distribution of solvent accessibility for all the twelve *Taq* polymerase structures with or without complex with DNA or Fab as described in Table 1. The *Taq*-Fab complex (1 BGX) is showing a high ASA (50000 $Å^2$) compared to other *Taq*-DNA complexes. Figure 13.6 shows the distribution of polar area and non polar area for all 12 *Taq* DNA polymerase structures. The overall structure contains more non-polar residues in the dataset. Figure 13.7 shows the solvent accessibility of residues in the native structure of *Taq* DNA polymerase with about more than 80% residues solvent exposed in the structure. Figures 13.8 to 13.15 show the interface residues in *Taq* polymerase when to Fab and or DNA complexes corresponding to the data in Table 13.1. They show that DNA and Fab bind to the polymerase domain (starting 419 and ending 832). Averages of 66 interface residues are involved in DNA or Fab binding (Table 13.2). Table 2 shows that about 84% of interface residues are hydrophilic and about 40% of them uncharged (standard uncharged residues are C, N, P, Q, S, T) in nature.

Taq DNA polymerase has an essential role in polymerase chain reaction for amplifying DNA fragments. The formation of *Taq* DNA polymerase complexes for catalysis and regulation is fascinating. The interaction between the *Taq* DNA polymerase and DNA or Fab is specific and sensitive (Table 13.1). The complete *Taq* DNA polymerase structures with its domains are shown in Figure 13.2. Figure 13.16 shows the crystal structure of *Taq* DNA polymerase in complex with an inhibitory Fab (TP7). The Fab is bound to the native enzyme's polymerase domain and it interacts with several residues that are usually involved in DNA binding (residue 515, 516, 536 and 540) (Murali *et al*. 1998). *Taq*-Fab in the complex form makes either van der Waals contacts or hydrogen bonds with these interface

residues. Hence, TP7 has the ability of binding to *Taq* polymerase and play its role as an inhibitory substance. These studies also reported a spontaneous reorientation of nuclease domain in *Taq* polymerase. This is due to the interaction between nuclease domain residues (260-290) and Fab residue (1L-26L).

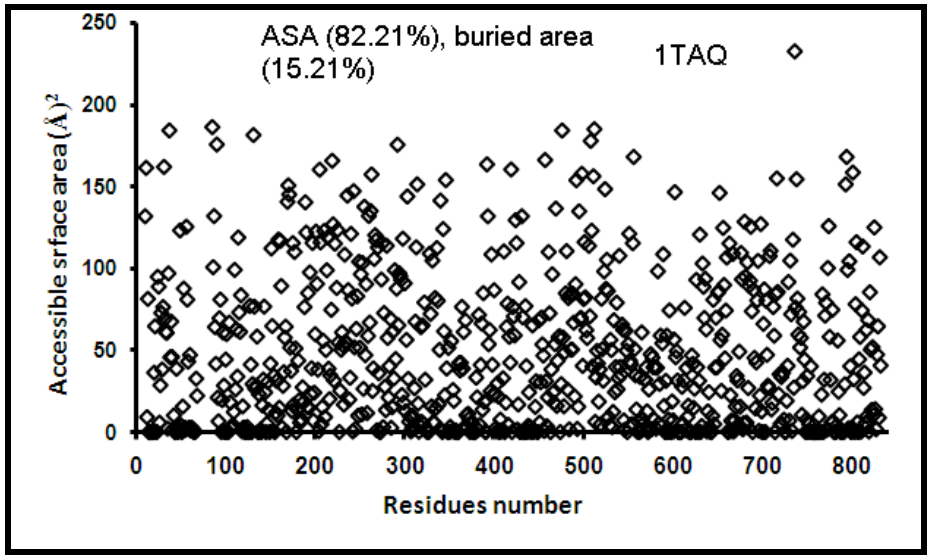

Figure 13.7. The distribution of accessible surface and buried surface area for the native *Taq* DNA polymerase (1TAQ) is presented in the above graph. This structure has 82.21% of accessible surface area and 15.21% buried area.

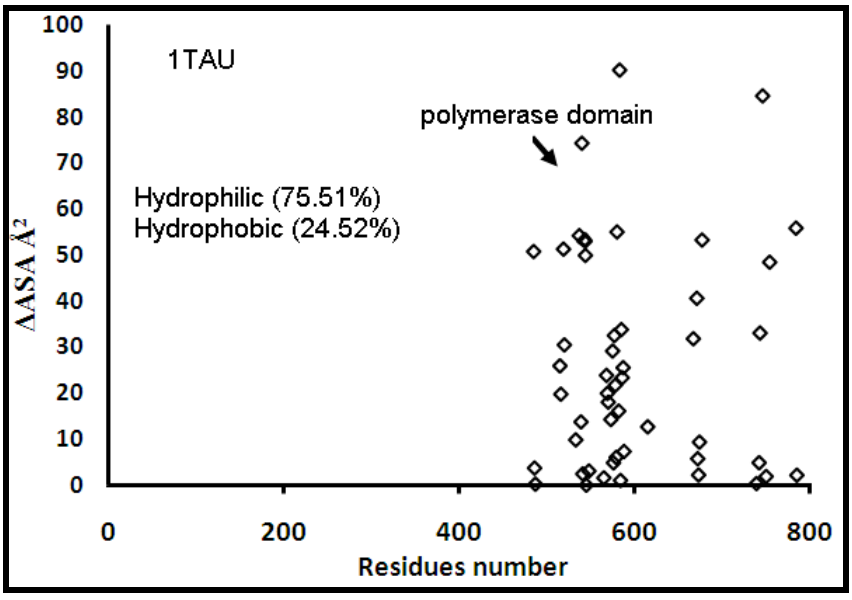

Figure 13.8. The delta ASA respective of residues number for the Taq polymerase with duplex blunt ended DNA (GCGATCCG) complex (1TAU). Only around 300(500-800) residues in the polymerase domain are involved in DNA binding. Though, ~50 residues directly interacting with the DNA. They are 75.51% hydrophilic and 24.52%. hydrophobic. (24.52%).

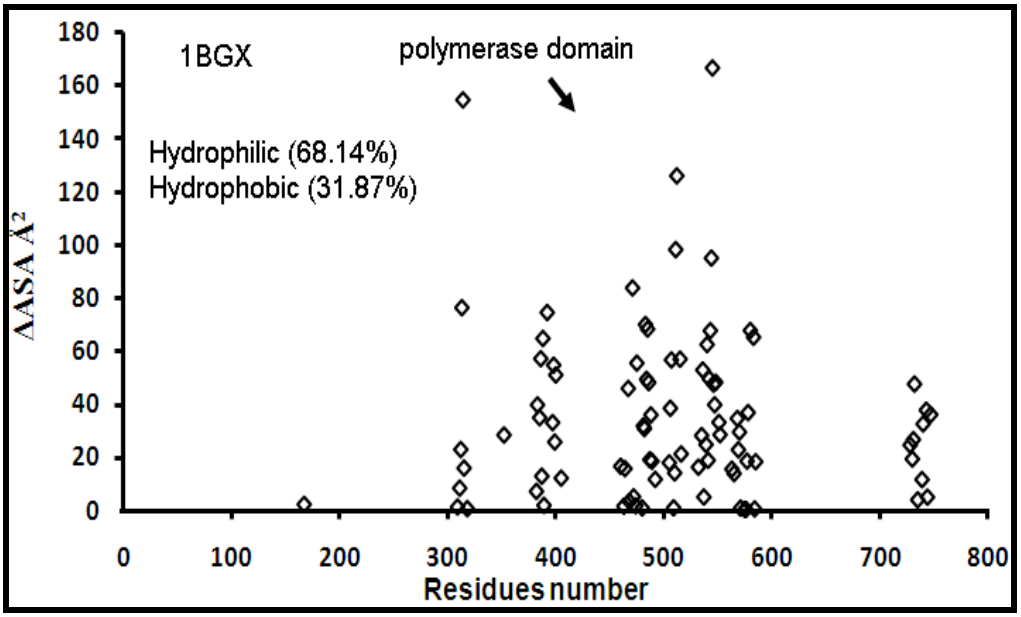

Figure 13.9. The distribution of delta ASA respective to residues number for Fab binding complex (1BGX). This complex has 91 residues which are interacting with Fab polymer. They are hydrophilic (68.14%) and hydrophobic (31.87%).

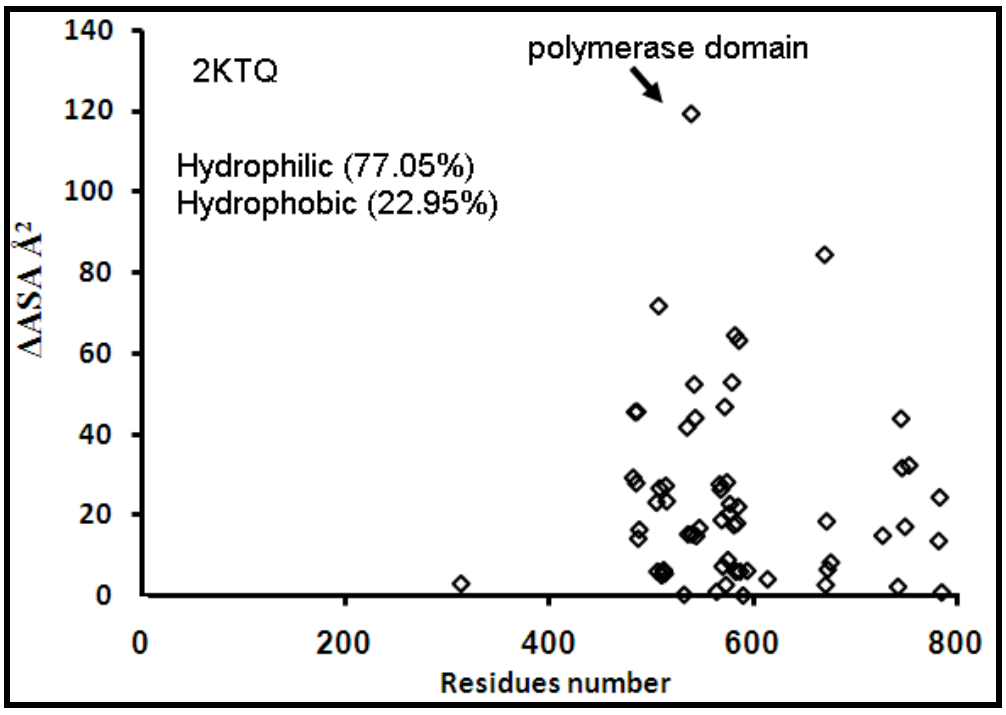

Figure 13.10. The distribution of delta ASA respective residues number for the open form ternary complex (2KTQ). 61 residues involve in DNA binding. They are hydrophilic (77.05%) and hydrophobic (22.95%) residues.

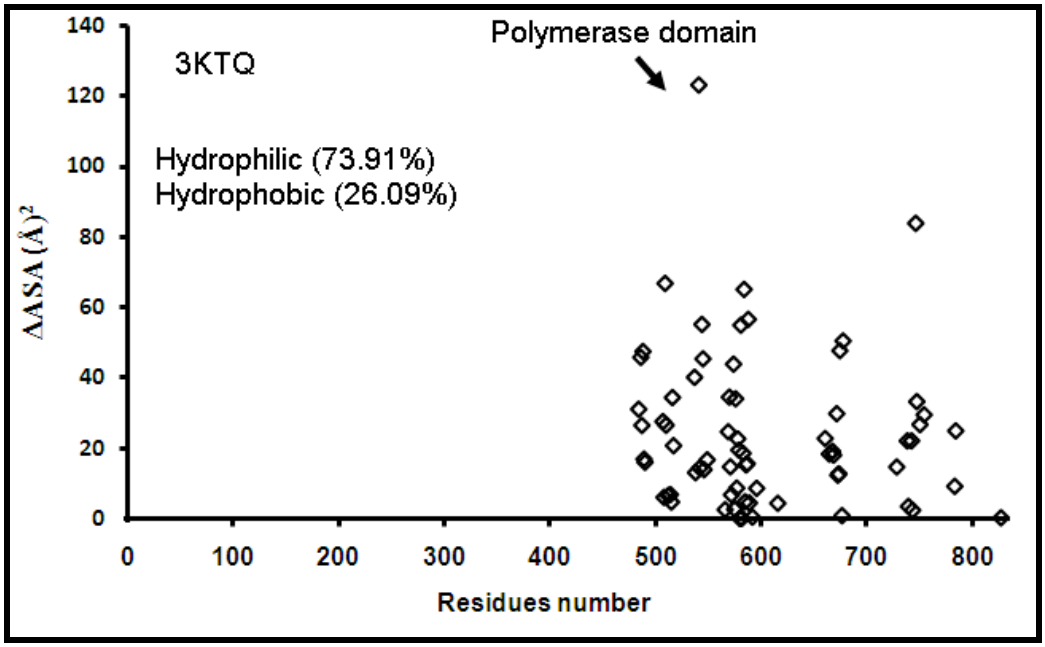

Figure 13.11. The distribution of delta ASA respective residues number for open binary complex (4KTQ). 58 residues involve in DNA binding. They are hydrophilic (79.31%) and hydrophobic (20.68%) residues.

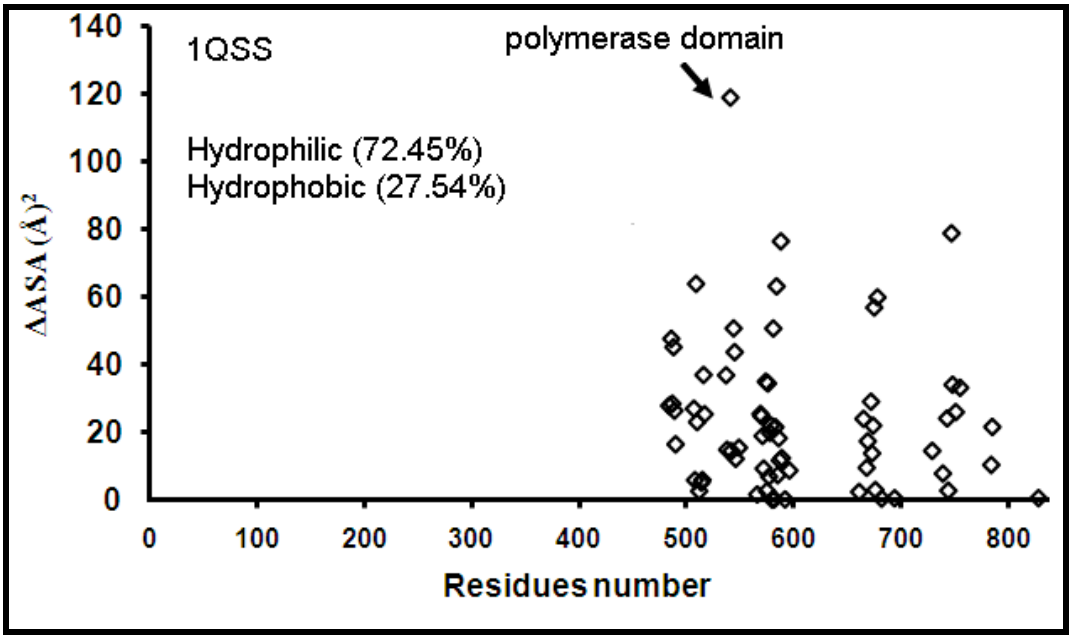

Figure 13.12. The distribution of delta ASA respective residues number for the closed form ternary complex (3KTQ). 69 residues involve in DNA binding. They are hydrophilic (73.91%) and hydrophobic (26.09%) residues.

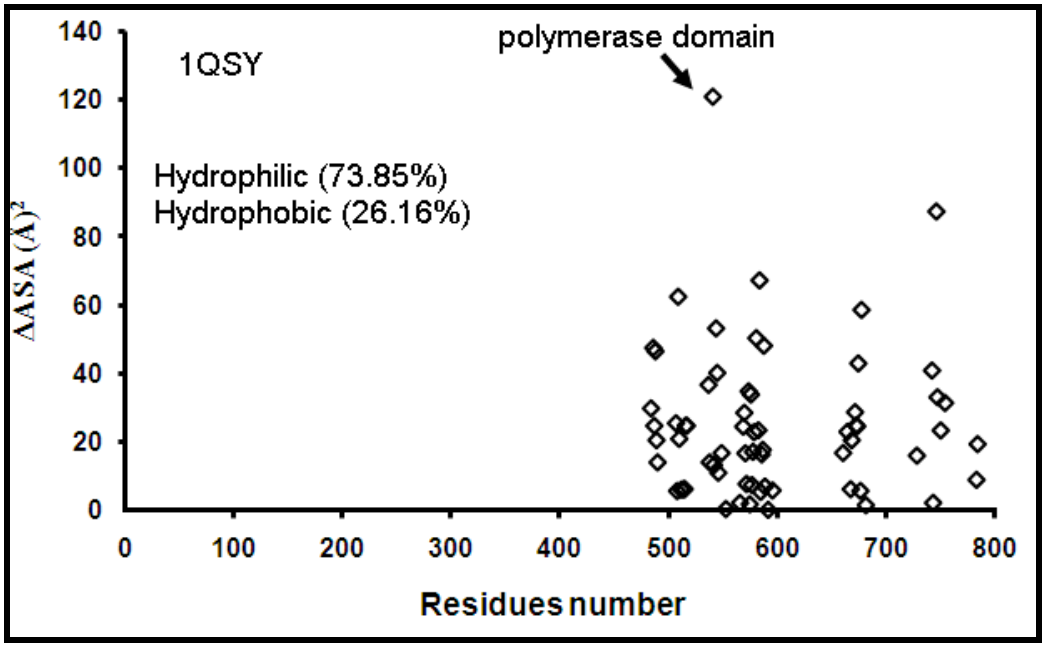

Figure 13.13. The distribution of delta ASA respective residues number for the ddGTP-trapped ternary complex (1QSS). 69 residues involve in DNA binding. They are hydrophilic (72.45%) and hydrophobic (27.54%) residues.

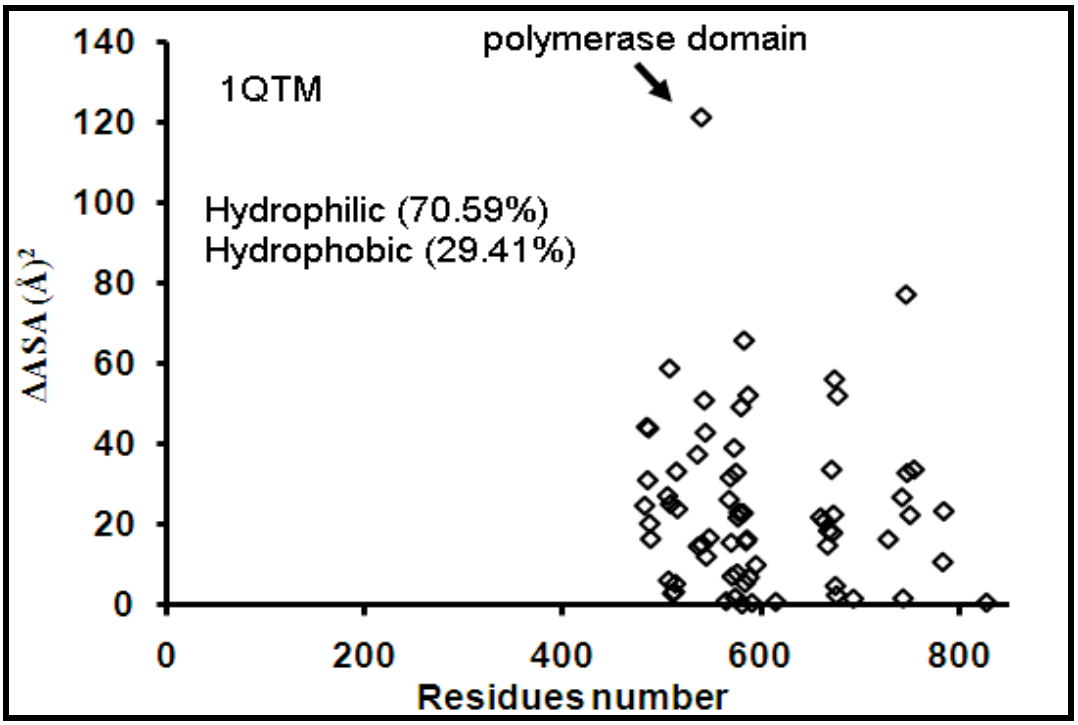

Figure 13.14. The distribution of delta ASA respective residues number for the ddATP-trapped ternary complex (1QSY). 65 residues involve in DNA binding. They are hydrophilic (73.85%) and hydrophobic (26.16%) residues.

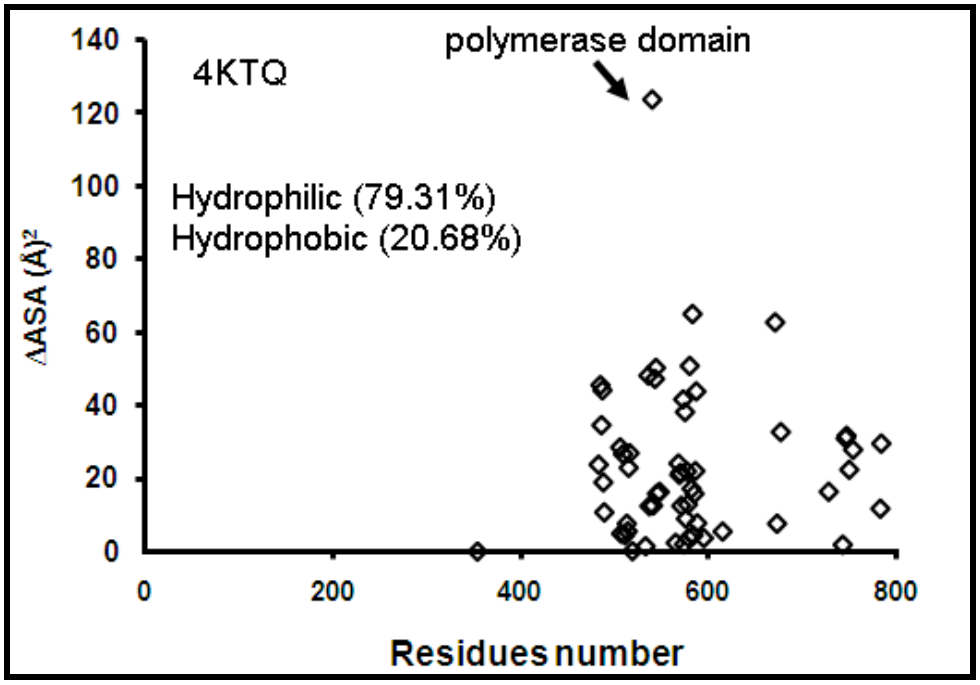

Figure 13.15. The distribution of delta ASA respective residues number for the ddTTP-trapped ternary complex (1QTM). 68 residues involve in DNA binding. They are hydrophilic (70.59%) and hydrophobic (29.41%) residues.

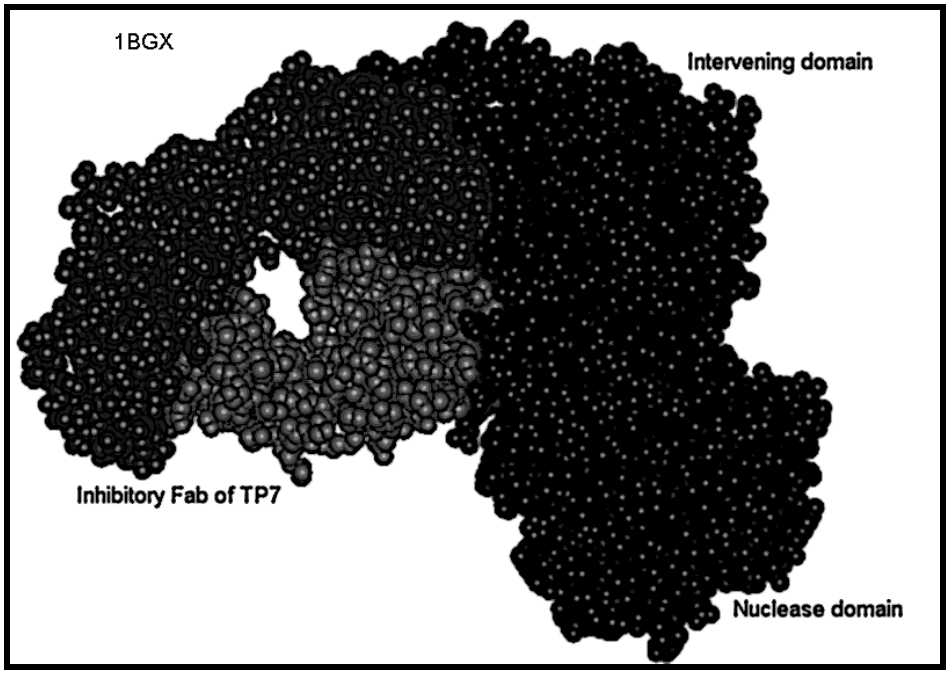

Figure 13.16. Crystal structure of *Taq* DNA polymerase in complex with and inhibitory Fab (PDB ID: 1BGX). The Fab they used was TP7. The TP7 antibody bound to the native enzyme as applied in hot-start PCR. Fab binding also causes the nuclease domain conformational change.

Li and co-workers (1998) determined the crystal structure of the open and closed forms of binary and ternary complexes of the large fragment of *Taq* DNA polymerase. Pelletier *et al.* (1994) studied the closed ternary complex consists of protein, DNA and ddCTP. Whereas the open ternary complex obtained by partial depleting of ddCTP component. And binary complex obtained by complete release of ddCTP. Figure 13.17 shows the closed ternary complex and Figure 13.18 shows the open ternary complex. These two structures are having two different orientations at the O-helix. Where, in the open form the Tyr 671 inserted in the stacking arrangement. Thus, Tyr 671 helps the DNA to bind at active site of *Taq* Polymerase. However, in the closed form the O-helix moved closer to the active site.Figure 13.19 shows the binary complex. Binary complex distinguished from the native enzyme in the change of thumb domain conformation (Korolev *et al*. 1995). The thumb domain rotating itself in two different angles (17° and 12°). The first rotation allowed the opening of a DNA crevice. The second rotation brings the helices (H1 and H2) closer to the DNA.

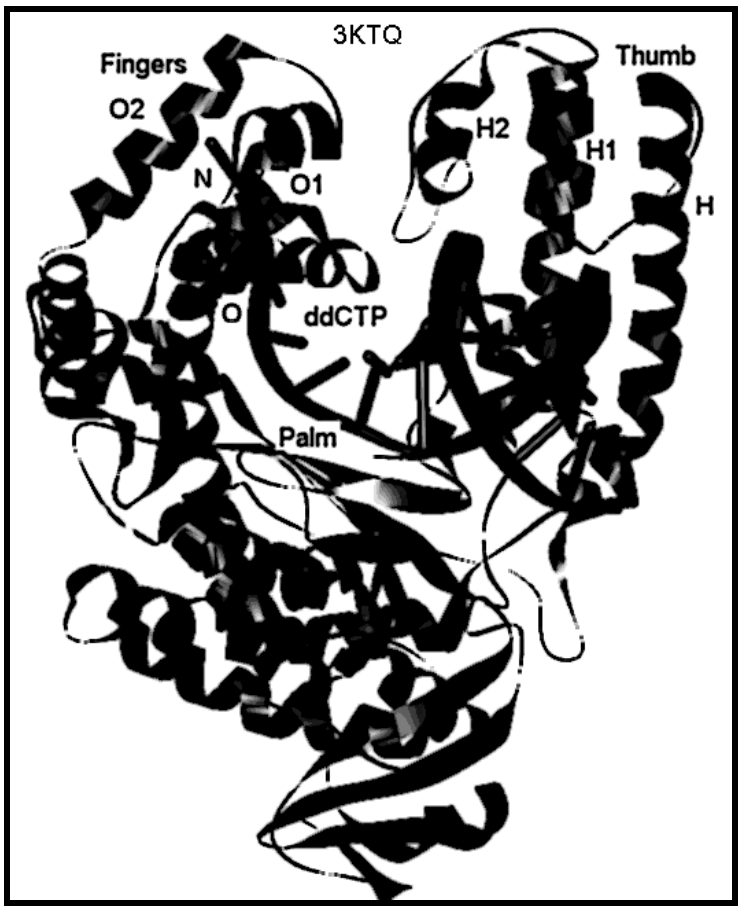

Figure 13.17. The closed form of ternary complex (PDB ID: 3KTQ). The first rotation (6°) of N, O, O1 and O2 results partial closing of crevice. Second rotation affects only N and O helix and cause the O-helix to move in closer to the active site.

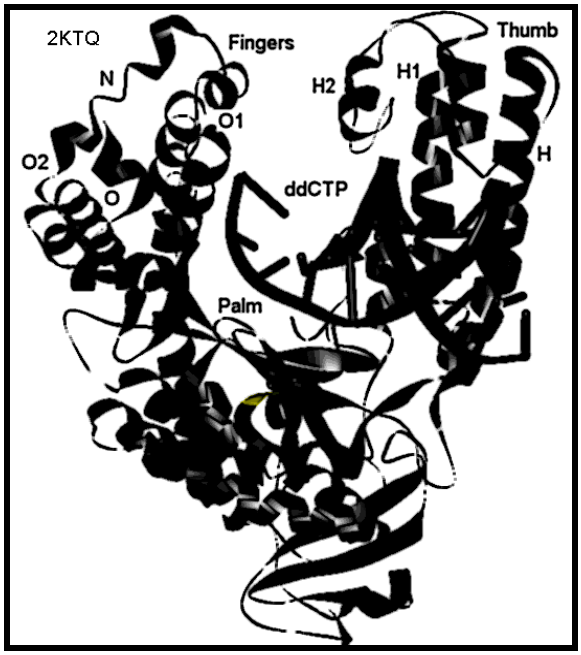

Figure 13.18. The open form ternary complex (PDB ID: 2KTQ). This complex trapped ddCTP component. Ternary complex consists of protein, DNA and ddCTP. This complex distinguished from other two complexes by large change in conformation of O-helix (O, O1 and O2) as well as the fingers domain.

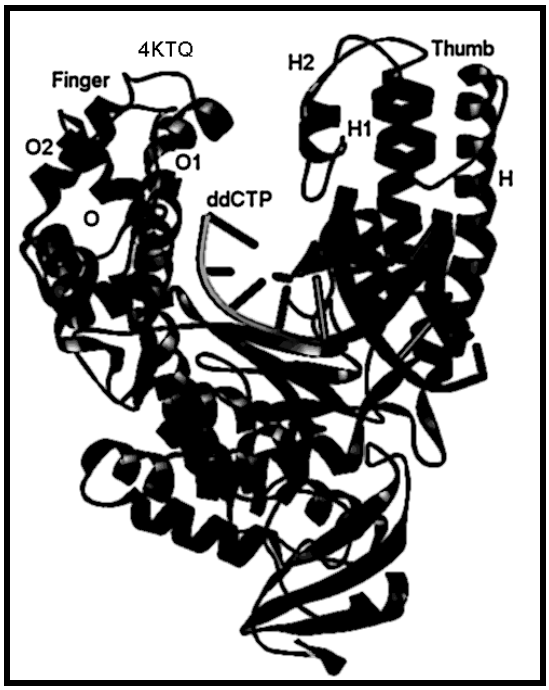

Figure 13.19. The open form binary complex trapped ddCTP (PDB ID: 4KTQ).The conformational change occurred in two parts. First thumb domain rotation (17°) results opening of active site. Second rotation (12°) brings the thumb domain (helices H1 and H2) closer to the DNA.

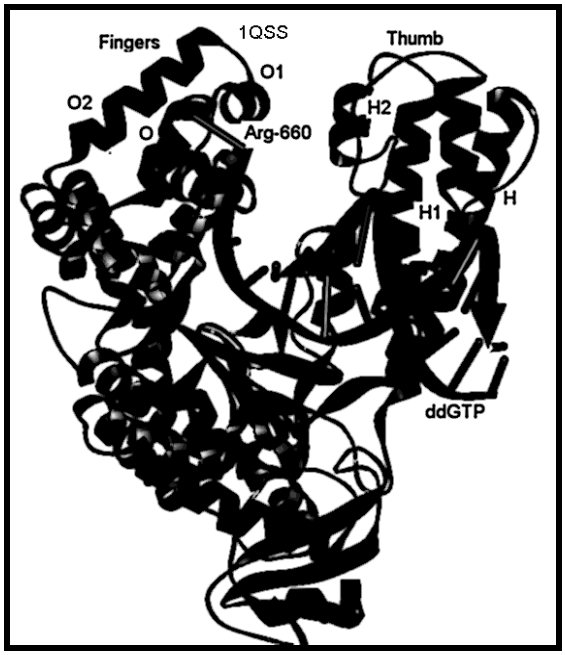

Figure 13.20. The ddGTP (dideoxyguanidine triphosphate) (PDB ID: 1QSS). This structure is distinguished from other three (ddATP, ddCTP, ddTTP). Arg-660 side chain formed hydrogen bond with the ddGTP base.

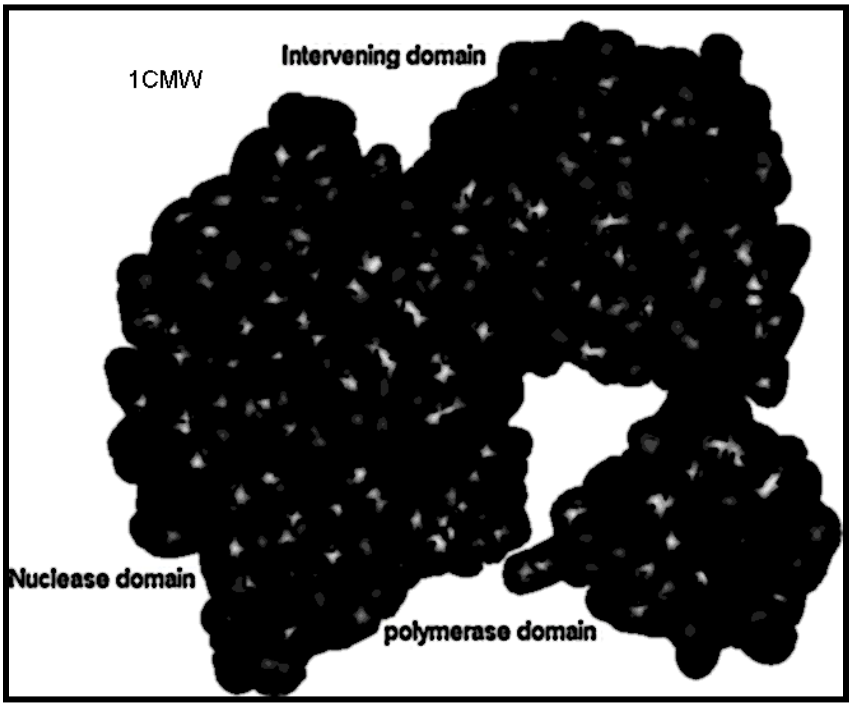

Figure 13.21. The new orientation of *Taq* DNA polymerase in compact form (PDB ID: 1CMW). This compact form has different nuclease domain orientation. Thus, the two active sites are considerably closer. This is allow the enzyme to catalyze polymerization and nuclease activities simultaneously.

Li and co-workers (1999) studied the structure of *Taq* DNA polymerase using dideoxynucleotide incorporation. They mutated *Taq* DNA polymerase at position 660. This causes the enzyme to incorporate the ddGTP at the rate similar to incorporation of ddATP, ddTTP or ddCTP. They proposed that the ddGTP-trapped complex differs from other three ternary complexes, because Arg-660 residue not interacting with the ddATP, ddCTP and ddTTP. Their structure also reveal that the higher ddGTP incorporation by the *Taq* polymerase is due to the selective interaction of residue 660 with the O6 and N7 atoms of the G base in the incoming ddGTP (Figure 13.20). Urs *et al*. (1999) have shown using anomalous diffraction using holmium edge technique that the structure of *Taq* DNA polymerase has a new orientation for the structure-specific nuclease. They found a compact form of *Taq* DNA polymerase which has different nuclease domain orientation. Thus, in this new orientation the two active sites are considerably closer. Thus, it can catalyze both, polymerization and nuclease activities simultaneously (Figure 13.21).

A comparative yet quantitative understanding of *Taq* DNA polymerase in complex with DNA and Fab is of interest. The interface properties between *Taq* DNA polymerase and Fab are documented. Figure 13.5 gives the distribution of solvent accessibility for all the twelve *Taq* polymerase structures with or without complex with DNA or Fab as described in Table 1. The *Taq*-Fab complex (1 BGX) is showing a high ASA (50000 $Å^2$) compared to other *Taq*-DNA complexes. Figure 13.6 shows the distribution of polar area and non polar area for all 12 *Taq* DNA polymerase structures. The overall structure contains more non-polar residues in the dataset. The result shows polar area and non polar area for all 12 *Taq* DNA polymerase structures. This implies that *Taq* DNA polymerase proteins have high non-polar area. Figure 13.7 shows the solvent accessibility of residues in the native structure of *Taq* DNA polymerase with about more than 80% residues solvent exposed in the structure.

It is our interest to document the physical-chemical characteristics of the interfaces between *Taq* polymerase with DNA and/or Fab (Figures 13.8 - 13.15). The DNA binding region is predominantly hydrophilic in nature for DNA (> 70%) and Fab binding (~ 68%). The Fab binds to Taq polymerase such that the activity of *Taq* is transiently inhibited for hot-start PCR (for improved specificity). Nonetheless, the Taq-Fab interface is also prevailingly hydrophilic (~68%). These observations have application in the design and development of better *Taq* DNA polymerase for effective PCR. Our observation suggests specific yet sensitive nature of protein-protein interactions in the case of *Taq* polymerase with the Fab molecule.

13.8. Conclusion

The *Taq* DNA polymerase from *Thermus aquaticus* is the most commonly used enzyme for *in vitro* DNA amplification using polymerase chain reaction (PCR). The enzyme finds application in molecular biology investigations and clinical diagnostics. Therefore, it is important to document the common structural features essential for DNA and protein binding with *Taq* DNA polymerase. We used *Taq* polymerase structures with Fab and DNA to glean essential information for better understanding. Our analysis show that the DNA binding region is predominantly hydrophilic (~ 74% in the dataset) in nature for DNA (> 70%) and Fab binding (~ 68%). The Fab binds to *Taq* polymerase such that the activity of *Taq* is

transiently inhibited for hot-start PCR (for improved specificity). Nonetheless, the Taq-Fab interface is also prevailingly hydrophilic (~68%). These observations have application in the design and development of better *Taq* DNA polymerase for effective PCR.

13.9. Exercises

[13.9.1] What is the application of *Taq* DNA polymerase?
[13.9.2] Expand PCR
[13.9.3] What are the steps involved in PCR?
[13.9.4] What is hot start PCR?
[13.9.5] Illustrate *Taq* DNA polymerase using a block diagram.
[13.9.6] How many domains are present in *Taq* DNA polymerase?
[13.9.7] What is the function of *Taq* DNA polymerase?
[13.9.8] How many structures have been solved for *Taq* DNA polymerase in complex form?
[13.9.9] Illustrate the interaction between DNA and *Taq* DNA polymerase?
[13.9.10] Illustrate the interaction between *Taq* DNA polymerase and Fab?
[13.9.11] What is the application of *Taq* DNA polymerase and Fab complex?
[13.9.12] What is the nature of residues at the DNA binding groove of *Taq* DNA polymerase?
[13.9.13] What is the nature of interface residues between *Taq* DNA polymerase and Fab?
[13.9.14] Describe the new orientation showed by Urs *et al.* (1999)?
[13.9.15] What is the function of the nuclease domain in *Taq* DNA polymerase?
[13.9.16] Which domain is responsible for polymerase activity in *Taq* DNA polymerase?
[13.9.17] Describe the intervening domain using a neat diagram?
[13.9.18] Name the group that solved *Taq* DNA polymerase structure for the first time?
[13.9.19] Expand *Taq*?
[13.9.20] Expand Fab?

13.10. References

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