relative extent of amyloid disaggregation appeared to be less than the decrease in crosslinking induced by PTB in tail-tendon collagen or red-cell-surface IgG. This may reflect a lesser contribution by AGE crosslinks to the physicochemical changes underlying βAP amyloid formation[12]. Electron microscopy revealed marked differences in the fibrillar structure of βAP aggregates before and after treatment with PTB (Fig. 4*b*). In control (untreated) preparations, β-amyloid was organized into dense fibrillar aggregates. The tubular-like fibrils were occasionally paired and showed a relatively uniform diameter of 9 ± 3 nm, a size consistent with that of β-amyloid fibrils *in vivo*[22]. By contrast, the preparation of βAP treated with PTB showed fibrils that were less dense, less uniformly organized, and more filamentous in form.

Our results support the hypothesis that a significant portion of the AGE crosslinks that form from glucose involve an α-diketone moiety that is susceptible to cleavage by thiazolium compounds such as PTB. Direct evidence for the formation of α-diketone crosslinks *in vivo* must await the isolation and complete characterization of these products. Although decreasing the AGE content of tissues may not necessarily ameliorate tissue pathology, this work does provide a rational basis for the design of AGE-cleaving agents that may ultimately be of broad clinical application. □

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Structure of *Taq* polymerase with DNA at the polymerase active site

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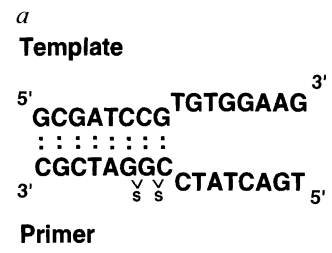
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THE DNA polymerase from *Thermus aquaticus* (*Taq* polymerase) is homologous to *Escherichia coli* DNA polymerase I (Pol I) and likewise has domains responsible for DNA polymerase and 5′ nuclease activities[1,2]. The structures of the polymerase domains of *Taq* polymerase and of the Klenow fragment (KF) of Pol I are almost identical, whereas the structure of a vestigial editing 3′–5′ exonuclease domain of *Taq* polymerase that lies between the other two domains is dramatically altered, resulting in the absence of this activity in the thermostable enzyme[2]. The structures have been solved for editing complexes between KF and single-stranded DNA[3,4] and for duplex DNA with a 3′ overhanging single strand[5], but not for a complex containing duplex DNA at the polymerase active-site. Here we present the co-crystal structure of *Taq* polymerase with a blunt-ended duplex DNA bound to the polymerase active-site cleft; the DNA neither bends nor goes through the large polymerase cleft, and the structural form of the bound DNA is between the B and A forms. A wide minor groove allows access to protein side chains that hydrogen-bond to the N3 of purines and the O2 of pyrimidines at the blunt-end terminus. Part of the DNA bound to the polymerase site shares a common binding site with DNA bound to the exonuclease site, but they are translated relative to each other by several ångströms along their helix axes.

Early models[6] of primer–template DNA at the polymerase active site had the DNA running through the cleft that was a distinctive feature of the KF structure. Numerous considerations, including the structure of an editing complex between KF and duplex DNA, as well as the location of completely conserved residues whose mutation affected polymerase activity[7], led to the conclusion that the duplex primer–template DNA approached the polymerase active site from the 3′–5′ exonuclease domain side of the cleft and perhaps was bent upon going through the cleft[5]. Based on comparisons between the structure of rat DNA polymerase-β (Pol-β) complexed with primer–template DNA and those of KF and HIV reverse transcriptase, it was suggested[8] that the primer–template approaches KF from the side opposite the 3′–5′ exonuclease domain, a conclusion that was disputed[9]. The crystal structure we describe here confirms the conclusion that the primer–template approaches the polymerase domain from the 3′–5′ exonuclease side of the cleft.

Taq polymerase was co-crystallized with two 16-mers of DNA that form 8 base pairs of duplex and 8 nucleotides of non-complementary single strands (Fig. 1*a*). Crystals diffract to 3.0 Å resolution at −165 °C using synchrotron radiation and are essentially isomorphous with those of the apo enzyme[2]. An electron density map (Fig. 1*b*), calculated from the protein coordinates alone but improved by cycles of density modification[10], shows good density for the bases and backbone of the duplex portion of the DNA.

The duplex DNA binds with its blunt end in the polymerase active site, packed against the O α-helix of the fingers domain and with its minor groove at the terminus interacting with side chains emanating from the palm domain (Fig. 2*a*). The DNA helix axis is roughly coincident with that of duplex DNA when bound in editing mode[5] and does not pass through the polymerase cleft (Fig. 2*b*). The 3′ hydroxyl of the primer strand is near to the three carboxylates (Asp 785[882], Glu 786[883], Asp 610[705]) that form the catalytic core of the polymerase active site, presumably by binding to divalent metal ions[7,8,11]. (Amino-acid sequence numbers of KF are given in brackets). The side chains of Tyr 671[766] and Phe 667[762], which are known to be important from their conservation in the Pol I family of polymerases[12,13] and from mutagenesis studies[13–16], are interacting with the bases at the blunt end of the duplex (Fig. 2*c*). Highly conserved amino-acid residues at the tip of the thumb (Asn 485[581], Ser 515[610], and



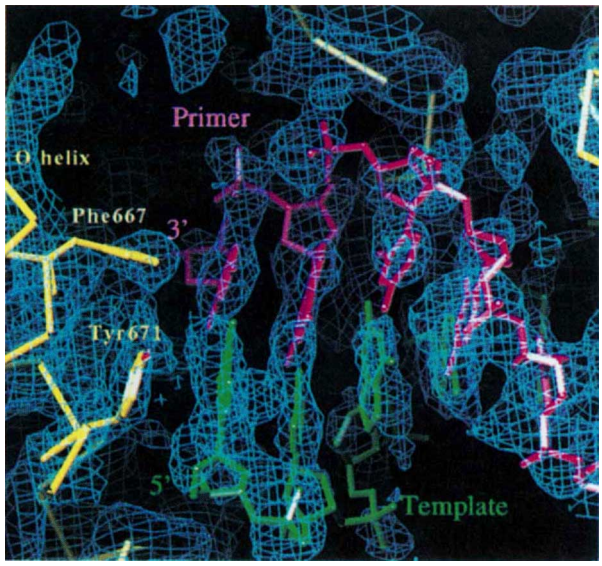


FIG. 1 Blunt-end DNA duplex co-crystallized with *Taq* polymerase. *a*, The DNA sequences used form 8 base pairs (bp) of duplex. Two nucleotides in the primer strand contained thiophosphates to prevent cleavage by the 5′ nuclease domain. The complex was crystallized at 22 °C in hanging drops containing 100 mM sodium citrate, 4% (w/v) polyethylene glycol 8000, 1 mM $ZnSO_4$, and 0–400 mM KCl at pH 6.0. Co-crystals were flash-frozen after transferring to a stabilizing solution containing 50 mM sodium citrate, 10% (v/v) glycerol, 15% (v/v) ethylene glycol, 100 mM KCl, 0.4% (w/v) β-octyl glucoside, and 10% (w/v) polyethylene glycol 8000 for 2 min. The co-crystals are isomorphous with those of the apo enzyme[2], belonging to space group $P3_12_1$ and having cell dimensions of $a = b = 107.6$, $c = 170.1$ Å. A data set to 3.0 Å resolution was collected at the X12C beam line at BNL ($\lambda = 1.00$ Å) and processed with DENZO and SCALEPACK with an overall merging *R*-factor of 8.8% (ref. 26). The crystallographic *R*-factor was 34% after rigid-body refinement of the protein coordinates alone and dropped to 28% after tightly restrained positional refinement using X-PLOR (ref. 27). Phases calculated form the refined protein structure were combined with the experimental phases of the apo *Taq* polymerase using SIGMAA weighting[28] to 3.0 Å resolution. Electron density maps calculated with these combined phases showed good density for the DNA bases but poor density for the backbone. To improve the low-resolution phases, which typically are of poor quality when calculated from the model, a procedure using cycles of density modification[10] to extend phasing from medium to low resolution was employed to improve the low-resolution phases. *b*, A 3-Å-resolution solvent-flattened electron density map resulting from the density modification procedure to improve the phases calculated from the coordinates of the protein in atoms alone shows good density for the bases as well as the backbone. The refined structure is superimposed. The 3′ base of the primer strand (pink) is interacting with Phe 667[762] on the O helix of the fingers domain and the 5′ base of the template strand (green) is interacting with Try 671[766]. Additional refinement including 8 bp of DNA reduced the *R*-factor to 23% and the free *R*-factor to 30%, with an r.m.s. deviation of bond lengths from ideality of 0.009 Å and bond angles of 1.5°, respectively.

Lys 540[635]) interact directly or indirectly with the DNA backbone across the minor groove (Fig. 2*c*). The small domain at the tip of the thumb lies over the 'top' of the DNA that is enclosed on three sides by the protein.

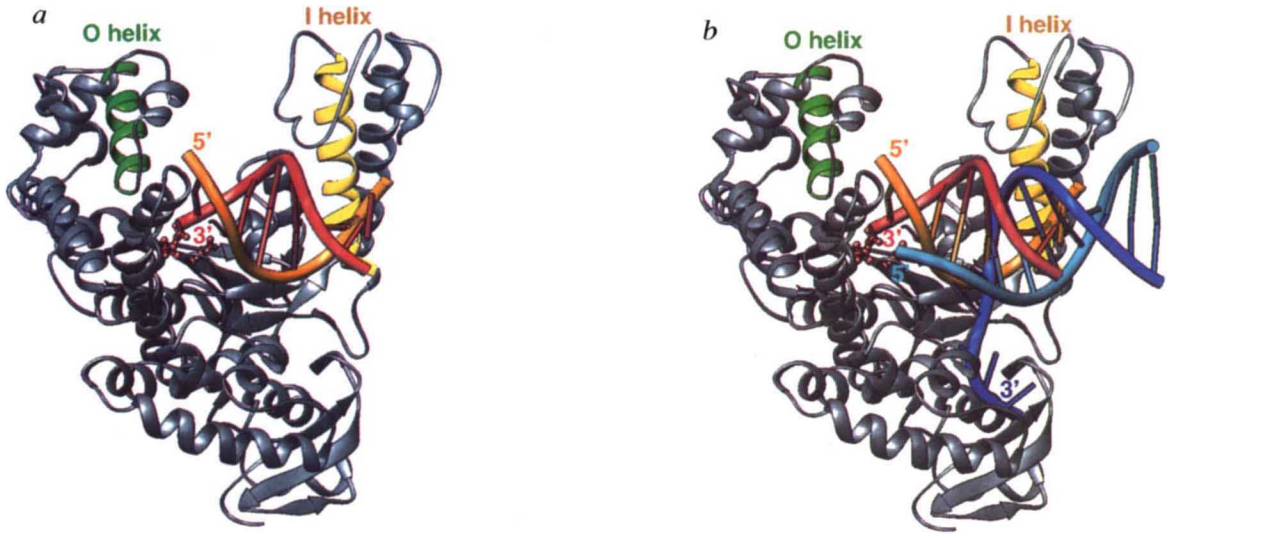
Most of the amino-acid residues in the polymerase domain that are highly conserved among the Pol I family[12,13] are in contact with or are very close to the bound duplex DNA (Fig. 2*c*), consistent with this complex being important in DNA synthesis. Seven absolutely conserved residues (Arg 573[668], Glu 655[710], Tyr 671[766], Asn 750[845], Gln 745[849], His 784[881] and Asp 785[882] contact the DNA (Fig. 2*c*). However, while the three catalytically important carboxylates are near to the 3′ nucleotide, they are too far away by 2–3 Å for metal ions bound to them to contact the 3′ hydroxyl group of the primer strand. The 3′ end of the duplex DNA observed here may lie further from the carboxylates than would be expected in a catalytically productive complex, due either to the lack of bound divalent metal ions or constraints on DNA positioning by crystal lattice interactions, or because it represents a distinct step in the polymerase reaction before or after the phosphoryl transfer step.

Comparison of the structure of the duplex DNA in this complex with those of A- and B-form DNAs shows that it has characteristics of both A and B form but is not identical to either (Fig. 3). Like A-form DNA, which has a groove width of 11.0 Å, the Pol I–DNA has a significantly wider minor groove (9.2 Å) than B-form DNA (5.7 Å) (ref. 17). Unlike A-form DNA, however, the base pairs are not highly tilted relative to the helix axis, nor are they significantly displaced outwards from the helix axis, which results in the bound DNA having a deeper minor groove than in A-form DNA. The DNA bound to Pol-β (ref. 8) has a similarly widened minor groove with characteristics of both A- and B-form DNA (Fig. 3).

Because the minor groove is wider than that of B-form DNA and faces the palm domain, it is accessible to protein side chains that can hydrogen-bond to the O2 atoms of pyrimidines and the N3 atoms of purines (Fig. 2*c*). These base hydrogen-bond acceptors lie in precisely the same place in the minor groove for all four base pairs[18]. It has been pointed out[19–21] that as these functional groups are invariant with sequence and differently positioned for non-Watson–Crick base pairs, their specific recognition and positioning by protein side chains may be an important mechanism by which polymerases enhance the fidelity of Watson–Crick base pairing and correct nucleotide incorporation at the polymerase active site.

This *Taq* polymerase–DNA co-crystal structure confirms the view[9] that the Pol I family of polymerases and HIV reverse transcriptase[22] bind primer–template to the polymerase active site located in the 'palm' subdomain in similar ways. Superposition of the Cα coordinates of the palm domains of these two polymerases puts the primer–template DNAs bound to the two enzymes in very similar orientations. The primer–template complex of *Taq* polymerase can be compared with that of DNA Pol-β by superimposing the bound DNA duplexes, as previously suggested[9]. This comparison shows many analogous interactions between the bound DNAs and these two non-homologous polymerases. In all three polymerases, the three catalytically important carboxylates of the palm domains are near the 3′-OH of the primer. Structurally unrelated helical protrusions (called 'thumb' in RT[23] and KF[3], and fingers in Pol-β[8] interact across the minor groove of the DNA product. The blunt end of a primer–template abutts a similarly positioned α-helix in the fingers domain of *Taq* polymerase and a non-homologous domain in Pol-β.

Comparison of the position of duplex DNA bound to the polymerase active site with that of DNA bound to the exonuclease active site (Fig. 2*b*) shows that although they share a common duplex DNA-binding site, the duplexes are translated relative to each other by several ångströms along their helix axes, resulting in different DNA-backbone interactions with the protein (Fig. 2*c*). It would appear that the duplex DNA binding site is constructed such that the DNA can slide by translation and may not be constrained to move only in a screw motion defined by the DNA backbone, a most surprising conclusion.



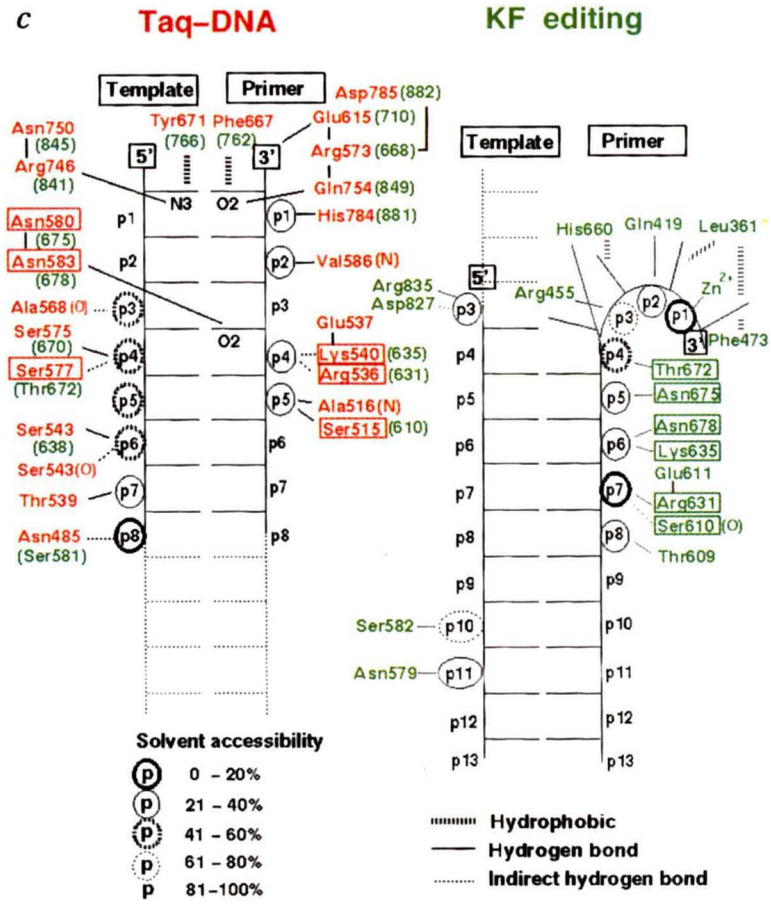


FIG. 2 The interaction of duplex DNA with *Taq* polymerase. *a*, Duplex portion of DNA on a ribbon representation of the KF portion of *Taq* polymerase. The O helix is in green, the I helix in yellow and the catalytically essential carboxylates, Asp 610[705], Asp 785[882] and Glu 786[883], are shown near the 3′ terminus. The template strand is orange and the primer strand is red. *b*, Superposition of DNAs bound in editing and in polymerizing modes. To orient the two DNAs, the polymerase domains of the KF and *Taq* polymerase complexes were superimposed and the DNA from the editing complex added to the *Taq* polymerase–DNA complex. Particularly striking is the difference in the positions of the primer backbones relative to the loop at the tip of the I helix. The template strand of editing-complex DNA is in cyan and its primer strand is in blue. *c*, Diagrams of the interactions between DNA and protein in the *Taq* polymerase-DNA and KF editing complexes showing that many of the same side chains interact with different phosphates in the two complexes. The names of *Taq* polymerase side chains are red and those of KF are green. Backbone carbonyl oxygen and amide nitrogen atoms are in parentheses. Thick dotted lines are hydrophobic interactions; thin lines, hydrogen bonds; thin dotted lines, indirect hydrogen bonds that might be through water molecules. Solvent accessibilities of the phosphate groups have been calculated using the program ACCESS[29]. Thick circles are 0–20% solvent-accessible; thin circles, 21–40%; thick dotted circles, 41–60%; thin dotted circles, 61–80%; blank, 81–100%. Side chains responsible for interactions and which interact in both complexes are boxed. The low solvent-accessibility of P5 of the template strand of the *Taq* polymerase–DNA complex results from Asp 578 (OD), Asp 578 (Cα), Pro 579 (Cα) and Asn 580 (N) lying within 4.5 Å. The coordinates used[5] to evaluate the interactions with the editing DNA were derived from a 3.2-Å-resolution map. As not all of the backbone and side-chain atoms at the tip of the thumb were located in this complex, the protein protection of the template strand is incomplete in this analysis. A 2.4-Å-resolution refinement of another editing complex is in progress (J. Jäger, D. Cahill and T.A.S., unpublished results).

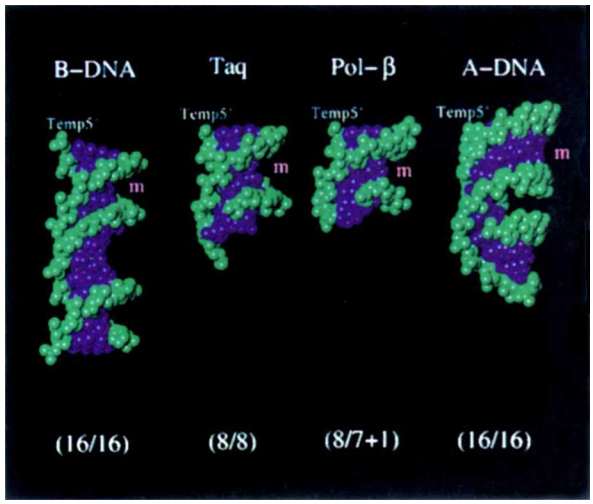


FIG. 3 Comparison of the structures of the duplex DNAs bound to *Taq* and Pol-β with those of A- and B-form DNAs. The coordinates of DNA in the Pol-β complex was taken from the Protein Data Bank[8]. The helix axes have been tilted by 32° to show the major and minor (indicated by m) groove. Bases are shown in blue; backbones are in green. The lengths of the template–primer strands are given in parentheses.

Comparison of these two complexes raises the question of how the 3′ terminus gets from one active site to another during the process of editing. It has been proposed[3,24], and supporting evidence obtained[25], that editing out misincorporated nucleotides is accomplished by competition for the 3′ end of the primer strand between the polymerase active site, which binds base-paired duplex DNA, and the exonuclease active site, which binds single-stranded DNA. As mismatched base pairs destabilize duplex DNA and promote the formation of single-stranded DNA, a mismatched primer terminus has a greater propensity to bind to the exonuclease site and be hydrolysed. The primer strand at its 3′ terminus lies between the template strand and the protein, and it appears not to be physically possible to move it to the exonuclease active site without the DNA translocating back. Whereas the DNA would not have to dissociate from the enzyme to go from one active site to another (indeed, in the homologous T7 DNA polymerase–thioredoxin enzyme it does not[30]), it appears that the 3′ end would have to dissociate from the polymerase active site and slide before entering the exonuclease active site. □

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CORRESPONDENCE and requests for materials should be addressed to T.A.S. The Brookhaven Data Bank accession numbers for the *Taq*–DNA complex and revised apo structures and their structure factors are 1TAU and 1TAQ, and R1TAUSF and R1TAQSF, respectively.

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