Hypothesis

A model for DNA polymerase translocation: worm-like movement of DNA within the binding cleft

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Abstract On the basis of recent results, we propose a model for DNA polymerase translocation along DNA. Human immunodeficiency virus reverse transcriptase is taken as an example. According to the model, movement of the enzyme is the result of transition of the enzyme-bound DNA from the A- to B-form which is accompanied by lengthening of DNA within the binding channel. The driving force of this transition is the increase in water accessibility to the DNA-binding cleft after dNTP binding. dNTP hydrolysis proceeding during the following chemical step supplies the energy for the reverse B→A transition of DNA. Translocation is considered to be an integral part of the stage of conformational change preceding catalysis and can be described as a worm-like movement of DNA within the DNA-binding cleft.

Key words: DNA polymerase translocation; HIV reverse transcriptase

1. Introduction

The progress achieved during the last decade in studies of the enzymes of template catalysis has allowed us to extend our understanding of their function on the molecular level. Recent investigations revealed striking similarities at the polymerase active sites [1–3] and led to generalized models for catalysis [1,2,4,5]. Nevertheless, the mechanism by which the DNA polymerases translocate along the template chain remains unknown [6]. Johnson [7] views translocation as a free diffusion of the polymerase along the DNA while in the open state. This free linear diffusion is described as an equilibration between the n and $n+1$ sites rather than as a distinct rate-limiting forward step of the polymerase along the DNA. However, numerous contacts between the amino acid residues of the enzyme and the nucleotides of DNA are observed in the resolved structures of DNA polymerases in opened complexes with dsDNA [8,9]. The free sliding model does not explain the process of breaking and restoring of these contacts. Patel et al. [6] believe that the enzyme moves down the nucleic acid in a ratchet-type manner, in 3.4-Å steps. The energy required for the movement of the protein could come from dNTP hydrolysis. In addition, the A- to B-form transitions of dsDNA may also supply some of the energy and dictate the overall direction of translocation. However, the authors do not discuss details of the mechanism, and its essence remains unclear. This mechanism does not explain at which step during the polymerization cycle the translocation occurs [6]. Several models for RNA polymerase translocation have also been proposed [10–15].

2. Our model

The model described below is based on the following experimental facts: (1) dsDNA within the binding channel of HIV RT is bent, and 6–7 base pairs near the 3′-end of primer are in the A-form [8]. Analogous structure of bound dsDNA is found in DNA polymerase β: the template primer assumes an A/B-form hybrid geometry [16]. Chimeric polynucleotides containing the regions with both A- and B-forms are known to be bent in the junction [17,18]. Therefore, the curved DNA-binding cleft is more suitable for accommodating the bent DNA. The bending of DNA within the binding channel is a common feature of many polymerases [9,19]. Besides, the high hydrophobicity of the cleft [20] is also favorable for the B→A transition [21]. It seems realistic to propose that such a transition is a typical phenomenon for DNA polymerases. (2) The same 6–7 base pairs near the 3′-end of primer are responsible for the specificity of the interaction between template and primer within the DNA-binding pocket of the enzyme [22]. (3) Many polymerases contain the 'helix clamp' structure which forms non-specific contacts with the internucleotide phosphates of the template primer [23]. In the case of HIV RT such a clamp consists of the αH and αI helices (Fig. 1) which contact the dsDNA at the point of A-B junction [8,23]. (4) DNA polymerases from different sources contain a Tyr residue near the 3′-end of the primer (Tyr-115 of HIV-1 RT [6] (Fig. 1), Tyr-766 of Klenow fragment [24], Tyr-865 of mammalian DNA polymerase α [25]). This residue is proposed to stack with the incoming dNTP [6]. (5) Conformational adaptation preceding the chemical stage of nucleotidyl transfer is a common step for many DNA polymerases and is thought to be responsible for polymerase fidelity [7,26–29]. Tryptophan fluorescence quenching studies reveal that this stage is accompanied by conformational rearrangement of the protein which changes from the opened to the closed state [7,26]. Another conformational change (the reverse transition) takes place after the chemical step [6,7,27]. Moreover, the enzyme undergoes conformational changes when dNTP binds to the polymerase in the absence of the template primer [30]. In this case, as well as upon dNTP binding to the enzyme-dsDNA complex, the fluorescence intensity sharply decreases.

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Abbreviations: dsDNA, double-stranded DNA; HIV RT, human immunodeficiency virus reverse transcriptase; araCMP, arabinosylcytosine 5′-monophosphate

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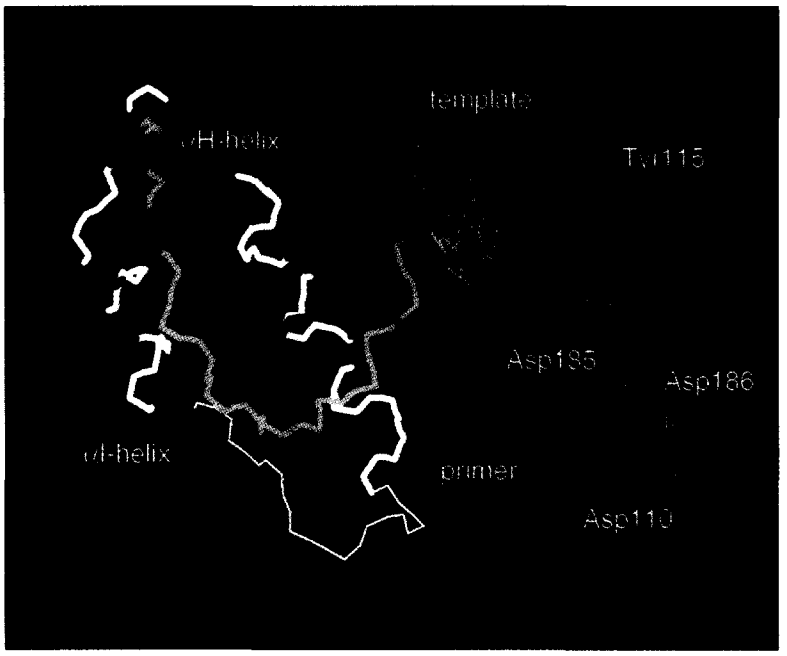

Fig. 1. Elements of the DNA-binding cleft and the dNTP-binding pocket. Red circles indicate the positions of Mg^{2+} ions. Catalytic residues of Asp-110, Asp-185, and Asp-186 are shown in green; Tyr-115 is shown in cyan. Incoming dTTP is colored in violet. The primer chain is shown in blue, the template chain in yellow. The helix clamp is drawn in white: the left thick white chain indicates the αI helix; the right thick white chain is the αH helix. The amino acid residues of αI and the internucleotide phosphates of the template chain, and, analogously, the amino acid residues of αH and the internucleotide phosphates of primer chain, which are involved in the formation of contacts between the enzyme and DNA before dNTP binding and incorporation [23], are shown as the red regions in these chains.

This reflects the increase in polarity of the microenvironment of the tryptophan residues. This is probably the result of the solvent-accessible surface of the enzyme being enlarged due to the translocation of individual subdomains.

3. Mechanism of translocation

For HIV RT we propose the following mechanism of translocation. When in the DNA-binding cleft, the template primer bends, forms contacts with the helix clamp, and undergoes a transition into the A-form in the region between the helix clamp and the 3′-end of the primer. Tyr-115 is not stacked onto the base of the 3′-end nucleotide at this stage (Figs. 1 and 2A). The triphosphate moiety of incoming dNTP first binds to the catalytic triad (Asp-110, Asp-185, and Asp-186), and its base stacks onto the Tyr-115 residue (in accordance with the model of Patel et al. [6], and with the data of Painter et al. [30] on the non-specificity of the initial dNTP binding). The bases of the entering dNTP and the 3′-end nucleotide are 3–4 Å apart [6]. dNTP binding leads to a conformational change of the enzyme and to an increase in its solvent-accessible surface. The latter leads to the A→B transition of DNA (increasing the extent of hydration is favorable for B-form DNA [21]). Conformational rearrangement of both the enzyme and DNA should weaken and/or partially break the protein-DNA contacts in the clamp. On the other hand, the A→B transition leads to a 6 Å extension of a 7 nt region of DNA duplex between the helix clamp and the 3′-end of the primer (from 18 to 24 Å) because the distance between the neighbouring nucleotides is 2.6 Å in A-form, and 3.4 Å in B-form DNA. This lengthening is likely to be a driving force moving the 3′-terminal nucleotide up to the base of the dNTP bound to the active site and to pull the duplex back 2–3 Å along the cleft using Tyr-115 as a fulcrum. Such a mechanism allows the simultaneous translocation of DNA and the spatial approach of the attacking 3′-OH group of the primer and α-phosphate of dNTP (the latter is the result of the stacking between the base of the 3′-terminal nucleotide and the base of dNTP) (Fig. 2B).

The enzyme is able to attain the critical catalytic configuration only if the newly formed base pair can adopt Watson-Crick geometry. If the dNTP is incorrect, the closed complex is unstable and easily breaks down upon the transition of DNA back to the A-form followed by dissociation of dNTP. The rapid transesterification step proceeds only after the closed state is stabilized. The hydrolysis of the dNTP provides the energy for the reverse B→A transition of the DNA region between the helix clamp and the newly formed 3′-end of the primer. This transition is thermodynamically and kinetically unfavorable in the closed state. The A-DNA restores weakened contacts with the helix clamp that leads to the conformational switching of the enzyme from the closed to the opened state, and the system returns to the starting structure, except for the following template position (Fig. 2C).

4. Crawling DNA

We would like to emphasize two principal features of the model described. First, translocation occurs simultaneously with the conformational adaptation of the substrate, i.e. it occurs before the incorporation of a new dNMP moiety. Second, conformational changes of the protein are not responsible for its translocation – instead, DNA 'crawls' along the binding cleft by means of lengthening and shortening, like a worm moving through the soil.

This mechanism can explain our earlier findings that the

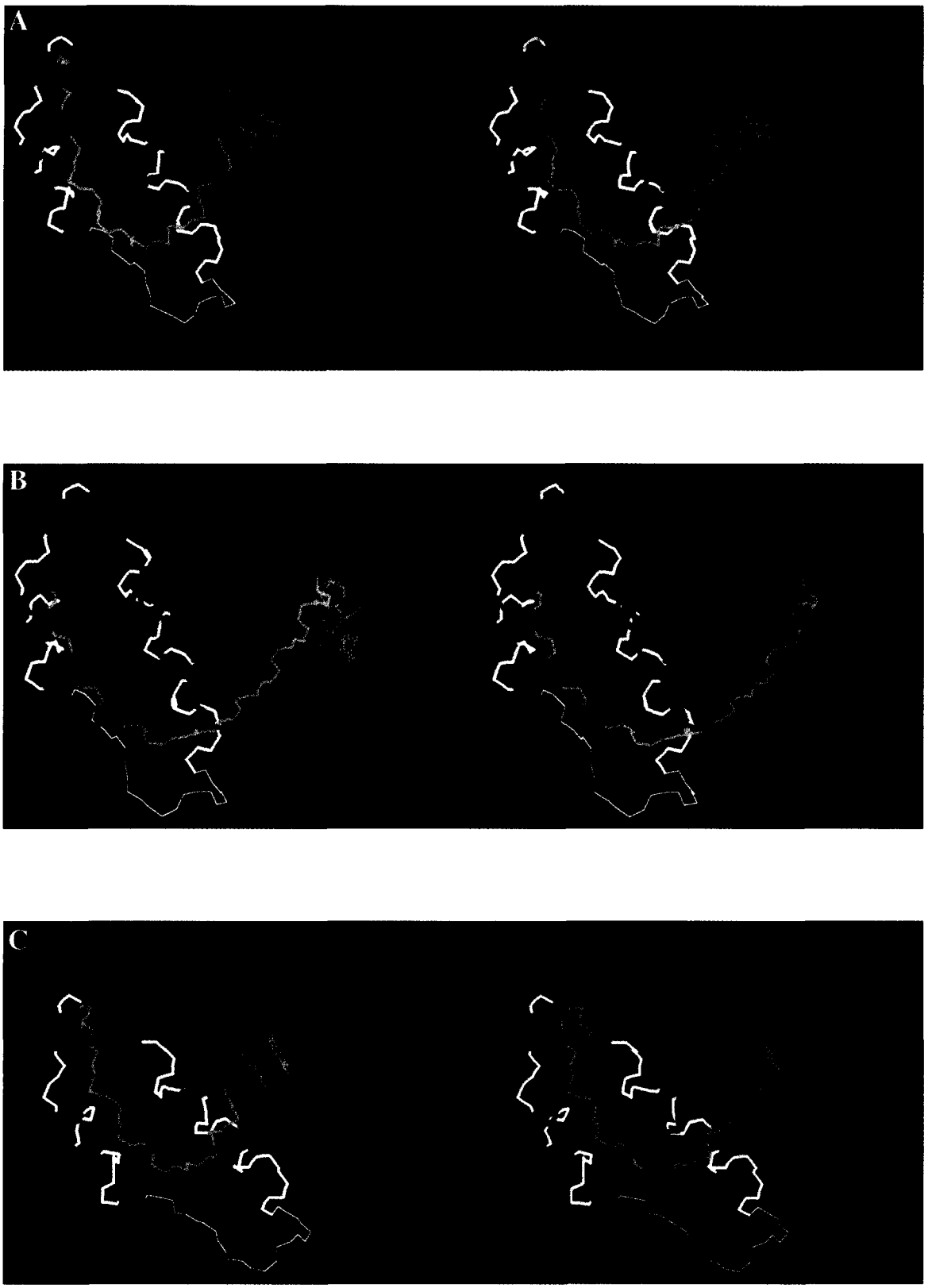

Fig. 2. Stereo diagram of the DNA-binding cleft and the dNTP-binding pocket. Data obtained from the Brookhaven Protein Data Bank (file 1HM1) were used for preparation of the figure. (A) Initial binding of dTTP. The triphosphate moiety is anchored by three Asp residues; the pyrimidine base is stacked with Tyr-115. DNA is in the A-form. (B) The stage of conformational rearrangement. DNA is in the B-form. The dTTP base is stacked with the 3′-terminal dA residue and forms the Watson-Crick pair with the dA residue of the template chain. It can be seen that the internucleotide phosphates, which were in contact with the helix clamp at the stage of initial binding, slide back and the marked residues of helix clamp can form the contacts with new positions of DNA. (C) The state of the system after the incorporation of the dTMP moiety. DNA is in the A-form. Red color indicate the sites which were interacting each other in A and became shifted after the incorporation of new nucleotide.

V_{max} values of DNA polymerization are sufficiently higher for many polymerases if ribooligonucleotides (or deoxyribooligonucleotides with the 3′-terminal ribonucleotide) have been used as a primer [31]. The A-like form is preferable for RNA-DNA hybrid duplexes [32,33] (and for some short duplexes which contains the ribonucleotide insertion [34]), hence the reverse B → A transition within the binding channel should have a lower energetic barrier in these cases.

Mikita and Beardsley [35,36] have shown that DNA replication can be arrested by structural lesion of arabinosylcytosine in the template DNA strand. A striking feature of this partial replication block is that it occurs at, rather than before, the template lesion site. There appears to be no impediment to the addition of the correct nucleotide, dGMP, opposite araCMP. Most other template lesions result in synthesis arrest one nucleotide before the lesion site because of gross distortion in the DNA structure and/or loss of the base-coding property ([35] and references herein). Structural studies on B-form DNA all indicate that the perturbations introduced by araC are slight to moderate in their effect, particularly compared to those changes introduced by many of the more bulky modifications of the DNA bases which grossly disturb duplex structure and stability. However, its inhibitory effects on DNA polymerase can be equal to or greater than some of these more aggressive structural lesions [36]. On the other hand, a molecular modeling study showed that strong steric clashes occurred when araC-G base pairs were built into an A-form helix environment [37]. On the basis of our translocation model, we could propose that the arabinosylcytosine lesion blocks the reverse transition from the B- to the A-form of DNA after the incorporation of a dGMP residue opposite the lesion site. As a result, the enzyme cannot restore the contacts, which are characteristic of the open state, and readily dissociates from the template primer.

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