Statement of teaching interests

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As a basic science, biochemistry is a discipline to describe the curious and wonderful phenomenon of life. It is based on the understanding of broad range of scientific knowledge, especially chemistry and biology. If undergraduate students have chances to get trained nicely in the field of basic sciences, it will be a great help to be contributors for the better world of mankind. I graduated from department of chemistry and got trained under the graduate program of biochemistry. Therefore I believe I have the qualification to teach undergraduate students general chemistry and biochemistry.

Although scientific knowledge of life has increased dramatically during the past decades, there still remain a lot of studies to be done. This drives outstanding students to take part in unveiling the questions of life. The undergraduate program should provide students with basic knowledge as well as motivation in life science. I will try my best to fulfill these goals.

For graduate program, I will focus on the understanding of enzymology. The graduate students usually take part in doing research in the laboratory, so that they need the basic knowledge of handling bio-macromolecules. The areas of protein purification, enzyme kinetics, protein-protein interaction, and protein-DNA interaction will be included in the major topics. I will also place great emphasis on the practical skills of experiments and data analyses. I hope students will eventually develop leadership in the field of biochemistry through my program.

Research summary : Kinetic analyses of nucleotide hydrolysis and DNA unwinding by Bacteriophage T7 helicase

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Helicases are motor proteins that translocate on DNA and catalyze separation of the dsDNA into ssDNA using the energy of NTP hydrolysis. This activity facilitates many nucleic acid metabolic processes during DNA replication, repair, and recombination (1-3). A class of helicases assembles into ring-shaped hexamers and hexamer formation is generally induced by NTP binding (4,5).

Bacteriophage T7 gp4A' protein assembles into hexameric ring in the presence of NTP, preferably dTTP. The translocation and separation activity of T7 helicase is fueled by dTTP hydrolysis. The hexamer has a high affinity for ssDNA that binds within the central channel of the ring (6). To unwind dsDNA, T7 helicase requires two noncomplementary tails at one end of the DNA. T7 helicase ring surrounds the 5' strand and excludes the 3' strand from its central channel during DNA unwinding (7).

In the laboratory of Dr. Smita S. Patel, I have been studying the mechanism of NTP hydrolysis and DNA unwinding by T7 helicase for the past four years. I will summarize the results so far, in which I have tried to answer very important topics.

(1) Kinetic pathway of dTTP hydrolysis in the absence of DNA

Previous study has shown that a model of dTTP hydrolysis by T7 helicase is similar to the binding change mechanism of F_1-ATPase with dTTP hydrolysis occurring sequentially at the catalytic sites (8). I examined in more detail the kinetic mechanism of dTTP hydrolysis by preassembled T7 helicase hexamer in the absence of DNA, where oligomerization did not limit the observed kinetics. Pre-steady state dTTP hydrolysis kinetics showed a distinct burst whose amplitude indicated that a preformed hexamer of T7 helicase hydrolyzes on an average one dTTP per hexamer. The pre-steady state chase-time experiments provided evidence for sequential hydrolysis of two dTTPs. The medium $[^{18}O]P_i$ exchange experiments failed to detect dTTP synthesis, indicating that the less than six-site hydrolysis observed is not due to reversible dTTP hydrolysis on the helicase active site. The P_i-release rate was measured directly using a stopped-flow fluorescence assay, and it was found that the rate of dTTP hydrolysis on the helicase active site is eight times faster than the P_i-release rate, which in turn is three times faster than the dTDP release rate. Thus, the rate-limiting step in the pathway of helicase-catalyzed dTTPase reaction is the release of dTDP. Chase-time dTTPase kinetics in the steady state phase provided evidence for two to three slowly hydrolyzing dTTPase sites on the hexamer.

(2) Sequential nucleotide hydrolysis in the presence of ssDNA

To understand the energy coupling from NTP hydrolysis to translocate along one of DNA strand, presteady state kinetic studies of T7 helicase in the presence of ssM13 DNA were performed. I have been collaborating with Dr. George Oster's group (UC Berkely) to better understand the dTTP hydrolysis mechanism by help of computational modeling. Transient and steady state biochemical measurements were analyzed using a numerical optimization scheme to compute the rate constants for the hydrolysis cycle and determine the flux distribution through the reaction network. The results show that (1) all subunits are catalytic; (2) the mechanical movement along the DNA strand is driven by the binding transition of nucleotide into the catalytic site; (3) hydrolysis is coordinated between adjacent subunits that bind DNA; (4) the hydrolysis step changes the affinity of a subunit for DNA allowing passage of DNA from one subunit to the next.

(3) dsDNA unwinding mechanism

T7 helicase binds preferentially to ssDNA within the central channel of the ring (9,10), a DNA binding mode that appears to be general to ring helicases. Here, I have studied the dsDNA unwinding mechanism of T7 helicase by measuring the single turnover kinetics. My results showed that T7 helicase unwinds DNA with a low processivity, and the results indicate that the low processivity is due to ring opening and helicase dissociating from the DNA during unwinding. Global fitting the data to a modified stepping model provided the kinetic step size, stepping rate, and proessivity. The comparison of the unwinding properties of T7 helicase with its translocation properties on ssDNA has provided insights into the mechanism of strand separation that is likely to be general for ring helicases. T7 helicase unwinds DNA with a rate of 15 bp/s, which is 9-fold slower than the translocation speed along ssDNA (11). T7 helicase is therefore primarily an ssDNA translocase that does not directly destabilize duplex DNA. I proposed that T7 helicase achieves DNA unwinding by its ability to bind ssDNA because it translocates unidirectionally, excluding the complementary strand from its central channel. The results also implied that T7 helicase by itself is not an efficient helicase and most likely becomes proficient at unwinding when it is engaged in a replication complex.

(4) Processivity of DNA unwinding

My previous dsDNA unwinding study showed that although the ring-shaped T7 helicase is inherently capable of traveling along kilo-bases of ssDNA, it shows poor processivity of DNA unwinding. Recently, through studies of the interactions of the displaced DNA strand with the T7 helicase, I have found that a link between unwinding processivity and the ability of T7 helicase to exclude the complementary DNA strand from its central channel. My results showed that T7 helicase unwinds DNA more efficiently when it cannot interact with the displaced complementary strand. Similarly, when the displaced complementary strand is sterically prevented from binding to T7 helicase by including ssDNA binding protein in the reaction a higher unwinding processivity is observed. Therefore I propose that the poor unwinding processivity of T7 helicase is

attributable to the tendency of the helicase ring to open during unwinding and occasionally bind the displaced complementary strand.

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Research plan : Mechanism of DNA replication by Bovine papillomavirus E1 and E2 proteins

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Papillomaviruses are small DNA tumor viruses that cause benign tumors in their hosts. All types of papillomaviruses induce persistent epithelial lesions. Upon infection, the viral genome enters the nucleus, where it is maintained as a multicopy plasmid. Some high-risk human papillomaviruses (type 16, 18, and 31) are known for causes of cervical carcinoma and low-risk types (type 6 and 11), although less critical, are responsible for sexually transmitted disease. One of the key processes in viral maintenance is DNA replication. Therefore better understanding of DNA replication is necessary for design of antiviral therapy.

Since the replication of Bovine papillomavirus (BPV) has been studied intensely, it is a very useful model system to study the papillomavirus DNA replication. BPV replication requires two viral proteins encoded by the E1 and E2 open reading frames (1). The E1 protein has a specific DNA binding activity to the viral origin (2-4), DNA helicase activity, and ATPase activity (5,6). To initiate DNA replication, E1 proteins bind the DNA binding sites. The second viral protein (E2) greatly enhances the DNA binding specificity of E1 through multiple protein-protein interactions (7). It is proposed that E1 has two separate DNA binding activities. One is through the DNA-binding domain (DBD) that has a high sequence specific activity, tethering to origin. The second is through the helicase domain that has a non-specific DNA binding activity. Although high specificity is achieved by DBD binding, the presence of non-specific DNA binding activity reduces overall specificity of E1 protein. This handicap could be overcome by the presence of E2 protein. E2 protein interacts with the helicase domain of E1 and prevents non-specific DNA binding. The E1-E2 complex binds DNA with high sequence specificity. However, in the presence of ATP, bound E2 recruits more E1 proteins and is displaced during the binding of additional E1 proteins to pre-existing E1 complex. Finally, further assembly of this complex with E1 proteins through unknown processes results in hexameric structure as shown in EM image analysis (8).

One of the proposed models suggests that BPV E1 protein acts as a bi-directional double hexamer, similar to SV40 T-antigen (9). Two hexamers are connected each other through interactions between the DBDs of the two hexamers. It is also proposed that each side of the initiator binding sites melts and then the extruded ssDNA triggers the DNA synthesis system in the presence of ATP. However, the exact mechanism of DNA melting and unwinding by E1 hexamer is not clear. In general, the initiation of DNA replication by helicase is poorly understood and how double hexamers initiate replication and continue on to catalyze unwinding in the elongation phase is not known, although several mechanisms have been proposed (10).

During my postdoctoral research in the laboratory of Dr. Smita Patel, I have studied the mechanism of NTP hydrolysis and DNA unwinding by T7 helicase. In my future research, I will study the molecular mechanism of DNA unwinding by the hexameric E1 helicase of BPV. T7 helicase is a single hexamer, so that my future research with double hexamer will be a new direction. To better understand the DNA replication mechanism by the hexameric E1 helicase, I will employ state-of-the-art pre-steady state kinetic methods and aim to dissect the parameters including the elementary rate constants and the equilibrium constants required to understand the molecular mechanism.

Specific Aims : DNA replication mechanism by BPV E1 hexameric helicase

The BPV type-1 E1 protein is a 68 Kda phosphoprotein that specifically binds to DNA at the viral origin of replication (11,12). The protein has an ATPase activity as well as a helicase activity that unwinds dsDNA. Although E1 alone shows specific binding to the viral origin, the binding activity is greatly enhanced by the E2 protein. After a multi-step assembly of E1, the E1 protein forms a bi-lobed double hexameric complex. It is proposed that the double hexamer unwind DNA bi-directionally, extruding the ssDNA from the rings. It is already known that single hexameric helicases (T7 helicase, *E. Coli* DnaB protein *etc*) bind preferentially ssDNA within the central channel of the ring and translocate along ssDNA, excluding the complementary strand from the ring. However, DNA melting process inside channel of the double hexamer is not clear. Does the double hexamer bind to dsDNA at multiple sites simultaneously and separate each strand? Next question is how the separated ssDNA is extruded from the ring after melting. Does the ring open and close so frequent that the ssDNA can be extruded?

To address these questions, I propose to conduct pre-steady state analyses of E1 hexamer with the following specific aims:

- *DNA unwinding mechanism of E1 hexamer.*

This study will include designing the best DNA substrate for unwinding as well as measuring the kinetic parameters. Of particular interest is how the dsDNA melting occurs inside the central channel of the hexameric ring. The mechanism of DNA exclusion from the ring will also be included.

- *Study of four-way (Holliday) junction branch migration by E1 hexamer.*

RuvB protein is known to have the ability of branch migration by double ring system. Previous studies proposed that E1 hexameic ring could bind dsDNA inside the channel. Based on the similarity for the DNA binding properties of both proteins, kinetic studies of branch migration will be conducted.

- *Protein-protein interaction between E1 and E2 proteins.*

E2 protein is known to enhance the DNA binding specificity by interaction with E1 proteins. However, E2 is displaced from the E1-E2 complex in the presence of ATP. This study will examine the properties of E2 quantitatively. I will also investigate the mechanism of E2 displacement and E1 hexamer formation.

- *Kinetics of ATP hydrolysis during dsDNA unwinding*

Helicases unwind DNA using NTP hydrolysis as energy source. Kinetic analyses of ATP hydrolysis will be examined during DNA unwinding by E1 hexamer. The energy coupling between ATP hydrolysis and unwinding movement will also be studied.

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