Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 341 (2006) 828–836
BBRC
www.elsevier.com/locate/ybbrc

Study of the ATP-binding site of helicase IV from *Escherichia coli*

Sandy Dubaele, Claude Lourdel, Patrick Chène *

Novartis Institutes for Biomedical Research, Oncology Department, CH-4002 Basel, Switzerland

Received 15 December 2005
Available online 23 January 2006

Abstract

Helicases contain conserved motifs involved in ATP/magnesium/nucleic acid binding and in the mechanisms coupling nucleotide hydrolysis to duplex unwinding. None of these motifs are located at the adenine-binding pocket of the protein. We show here that the superfamily I helicase, helicase IV from *Escherichia coli*, utilizes a conserved glutamine and conserved aromatic residue to interact with ATP. Other superfamily I helicases such as, UvrD/Rep/PcrA also possess these residues but in addition they interact with adenine via a conserved arginine, which is replaced by a serine in helicase IV. Mutation of this serine residue in helicase IV into histidine or methionine leads to proteins with unaffected ATPase and DNA-binding activities but with low helicase activity. This suggests that residues located at the adenine-binding pocket, in addition to be involved in ATP-binding, are important for efficient coupling between ATP hydrolysis and DNA unwinding.

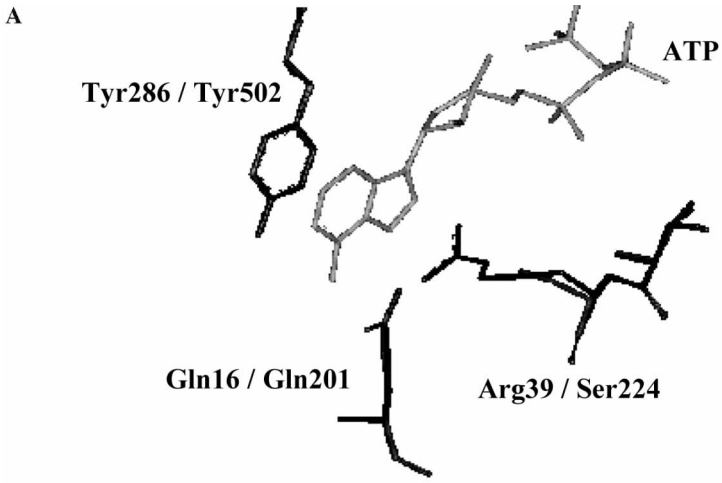
Keywords: Helicase; Helicase IV; Q motif; ATPase; Uncoupling

Helicases are ubiquitous enzymes involved in nucleic acid metabolism [1]. They utilize the energy liberated during the hydrolysis of nucleotides (NTP) to catalyze the separation of double-stranded nucleic acids [2]. Several motifs are conserved amongst all helicases. They can be roughly grouped into three categories: motifs that interact with NTP and the magnesium ion co-factor; motifs that are involved in the interaction with the oligonucleotide; and motifs that participate in the molecular mechanisms involved in coupling the NTPase cycle to strand displacement and duplex unwinding [3,4]. The study of the structure of helicases reveals that the conserved motifs involved in NTP binding are located within the phosphate-binding site but not at the adenine-binding pocket [3]. This is expected for two reasons. First, the presence of a γ-phosphate at the active site might be important in coupling ATP hydrolysis to strand separation. Residues involved in this process are thus key for helicase activity and must be conserved. Second, enzymes have evolved to be more complementary to the transition state than to substrate. Therefore, residues involved in the breakage of the β–γ phosphate bond must be well conserved. Recently, one motif—the Q motif—has been described as a conserved element in the adenine-binding pocket of DEAD-box RNA helicases [5]. It has been proposed that this motif could be used as a sensor of the bound nucleotide that is used to regulate RNA-binding and helicase activity [5,6]. This finding suggests that the region surrounding adenine, in addition to interacting with ATP, plays additional roles in the catalytical activity of helicases.

To study the role of residues located within the adenine-binding pocket of helicases, we focused our attention on superfamily I helicases because the structure of two members of this superfamily—PcrA from *Bacillus stearothermophilus* and Rep from *Escherichia coli*—in complex with a nucleotide is available [7–9]. In PcrA from *B. stearothermophilus*, the adenine moiety is in stacking interaction with the phenyl ring from Tyr286 and is close enough to Gln16 to make hydrogen bonds via N6 and N7 (Fig. 1A). Primary sequence comparison amongst different helicases from the UvrD/Rep/PcrA family shows that Tyr286 and Gln16 are well conserved (Fig. 1B), suggesting that these residues are important for the activity of these

* Corresponding author. Fax: +41616963835.
E-mail address: patrick_chene@yahoo.com (P. Chène).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.01.040



B

UvrD/Rep/PcrA	%	<--MI--> *	
PcrA *B. stearothermophilus*	hlnke**Q**qea	agagsgkt**R**vlt	eqn**Y**rst
PcrA *L. citreum*	gmnnk**Q**aea	agagsgkt**R**vlt	eqn**Y**rst
PcrA *S. aureus*	hmnte**Q**sea	agagsgkt**R**vlt	eqn**Y**rst
Rep *E. coli*	rlnpg**Q**qqa	agagsgkt**R**vit	eqn**Y**rss
Rep *H influenzae*	klnpq**Q**qqa	agagsgkt**R**vii	eqn**Y**rst
Rep *S. typhimurium*	rlnpg**Q**qha	agagsgkt**R**vit	eqn**Y**rss
UvrD *E. coli*	slndk**Q**rea	agagsgkt**R**vlv	eqn**Y**rst
UvrD *H. influenzae*	slndk**Q**rer	agagsgkt**R**vlt	eqn**Y**rst
UvrD *S. typhimurium*	glndk**Q**rea	agagsgkt**R**vlv	eqn**Y**rst
Helicase IV			
Hel4 *E. coli*	plnpa**Q**ara	agagsgkt**S**vlv	dtt**Y**rfn
Hel4 E. carotovora	plnms**Q**ska	agagsgkt**S**vlv	dtt**Y**rfn
Hel4 S. enterica	plnps**Q**ara	agagsgkt**S**vlv	dtt**Y**rfn

Fig. 1. Adenine-binding site of UvrD/Rep/PcrA helicases and helicase IV. (A) Overlay of the amino acids located at the adenine-binding site of PcrA from *B. stearothermophilus* and of helicase IV from *E. coli* at the ATP-binding site. (B) Primary sequence alignment of 3 PcrA, 3 Rep, 3 UvrD, and 3 helicase IV proteins. The conserved glutamine (Q), arginine (R), tyrosine (Y), and serine (S) residues are represented in capital letter and in bold. The star indicates Arg39 in PcrA from *B. stearothermophilus* and Ser224 in helicase IV from *E. coli*. % indicates the position of Pro196 in *E. coli*. The position of motif I (MI) is indicated.

enzymes. The interactions made by these two residues are similar to the ones described in DEAD-box RNA helicases (Q motif) [5]. However, in DEAD-box RNA helicases the conserved glutamine and phenylalanine are both located upstream motif I while in UvrD/Rep/PcrA helicases the glutamine and the tyrosine residues are located, respectively, upstream and downstream of motif I. There is an additional difference between both types of enzymes. In Rep and PcrA, the guanidinium moiety from an arginine residue, Arg39 in PcrA from *B. stearothermophilus*, interacts with the purine ring (Fig. 1A). This arginine residue is conserved amongst UvrD/Rep/PcrA helicases (Fig. 1B).

Interestingly this additional interaction is not present in all DNA helicases belonging to superfamily I. For example helicase IV proteins, which are also superfamily I members [10], do not possess an arginine residue at this position but a serine (Fig. 1B). Since this residue is presumably too short to interact with the adenine moiety (Fig. 1A), helicase IV proteins may only contact adenine via their conserved glutamine and tyrosine, as do DEAD-box RNA helicases. Replacing this serine by amino acids with a longer side chain may affect ATP-binding and as a consequence informs us on the influence of an additional interaction with adenine in the activity of these enzymes. In this report, we study the effect of mutations of different residues located at the adenine-binding pocket of helicase IV from *E. coli* on its ATPase and helicase activity.

Materials and methods

Cloning and mutation of helicase IV. Genomic DNA from *E. coli* strain BL21 (Stratagene) was obtained with the Wizard Genomic DNA purification kit (Promega) following the instructions provided by the manufacturers. The gene encoding for helicase IV was isolated by polymerase chain amplification (PCR), and *Nde*I/*Bam*HI restriction sites were introduced at its 5′ and 3′ ends, respectively. The two PCR primers were designed according to the DNA sequence published by Wood and Matson [18]. The amplified gene was introduced into the *Nde*I/*Bam*HI sites of a pET-15b vector (Novagen).

Site-directed mutagenesis. Site-directed mutagenesis of the helicase IV gene cloned in pET-15b was performed by PCR using the QuickChange XL site-directed mutagenesis kit (Stratagene) according to manufacturer's instructions. The mutated state of all the cDNA constructs was confirmed by double strand sequencing (Solvias AG).

Protein expression and purification of the helicase IV proteins. The different expression vectors were transformed into *E. coli* BL21(DE3)-pLysS cells (Stratagene). Individual colonies were selected on ampicillin