Nucleotide and DNA binding to a DnaB helicase studied by solid-state NMR

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DnaB helicases are bacterial, ATP-driven enzymes that unwind double-stranded DNA into their single-stranded analogues as a key step during the DNA replication process[1]. The detailed mechanism of this process differs from organism to organism and in many cases atomic-resolution structural data are not accessible since the formed complexes of the helicase with nucleotide and/or DNA escape crystallization and therefore X-ray diffraction as an essential structure determination technique.

In these cases, solid-state NMR is besides electron microscopy (EM) a convenient choice since the proteins can be studied as a sediment formed in the Magic-Angle Spinning (MAS) rotor[2]. In this contribution, we study the helicase-nucleotide and helicase-DNA binding of the dodecameric DnaB helicase from Helicobacter pylori[3]. In a first step, the complex between DnaB, MgCl₂ and the non-hydrolysable ATP-analogue Adenylyl-imidophosphate (AMPPNP) is characterized by 2D and 3D solid-state NMR experiments. Chemical-shift perturbations (CSPs) were used to monitor the AMPPNP binding and to identify the binding interaction interfaces, as well as to determine overall structural changes in the helicase.

In a second step, a polythymine stretch consisting of 20 nucleic acids is added to the helicase-AMPPNP-MgCl₂ complex to mimic the binding of single-stranded DNA (ssDNA). The formed complex is studied by solid-state NMR experiments. ³¹P CPMAS-NMR experiments are performed additionally for proving the binding of ssDNA, as well as the ATP-analogue to the helicase.

HpDnaB

Literature