Mechanism of action of DNA gyrase

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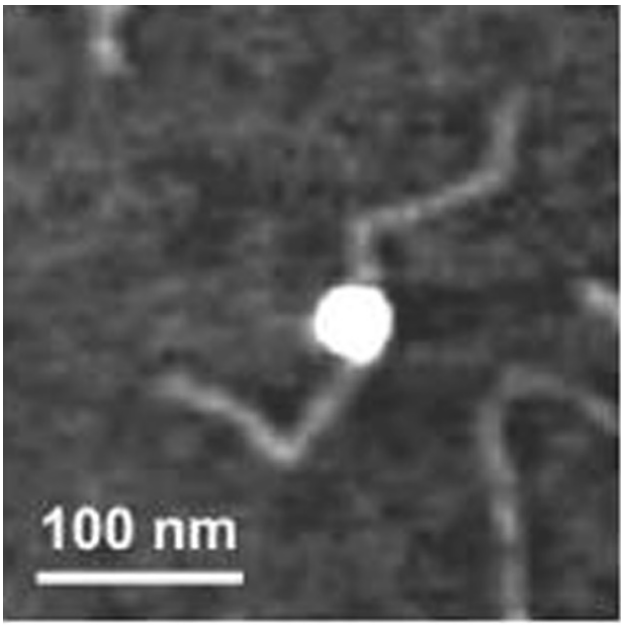
DNA gyrase is a bacterial motor protein in a class known as topoisomerases, which are responsible for controlling the topological properties of DNA (e.g. amount of supercoiling or catenation). Most topoisomerases can relax supercoiled DNA, which is an energetically favourable process. DNA gyrase is unique amongst this class, because it can introduce supercoils as well as remove them. To wind or unwind DNA it must break both strands of DNA, capture another segment of the same DNA molecule and pass this through the double-strand break before resealing. However, it can only introduce supercoils with a given chirality: i.e. negative supercoils. It is thought to achieve this by wrapping DNA around itself in a right-handed sense, which determines the direction of strand capture and passage. It needs the free energy release of ATP hydrolysis to drive this energetically unfavourable process. Previous evidence from DNA footprinting assays and EM analysis has indicated the presence of this wrap. Measuring DNA contour lengths from tapping-mode AFM images of gyrase bound to DNA, we have confirmed that gyrase wraps between 90 and 150 base-pairs of DNA. On addition of ADPNP (the non-hydrolysable analog of ATP) to the complex, which can support one round of supercoiling, this wrap is lost. The AFM data for this has been confirmed over a wide range of conditions using a topoisomerase I relaxation assay. Previous biochemical and biophysical experiments have given conflicting results, indicating both an increase and loss of wrap upon addition of ADPNP. We are developing a revised model of DNA strand passage by DNA gyrase that takes all these data into account.



Tapping-mode AFM image in air of DNA gyrase bound to a 1070bp dsDNA template in the absence of ADPNP

Collaborators

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