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RNA HELICASES IN ACTION

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The *Eschericia coli* protein, DbpA, is unique in its subclass of DEAD box RNA helicases because it possesses ATPase specific activity toward the peptidyl transferase center in 23S rRNA. Although its remarkable ATPase activity is well defined toward various substrates, its RNA helicase activity remained to be characterized. Here we show by using biochemical assays and Atomic Force Microscopy (AFM) that DbpA exhibits ATP-stimulated unwinding activity of RNA duplex regardless of its primary sequence. This work presents an attempt to investigate the action of DEAD-box proteins by a single-molecule visualization methodology. Our AFM images enabled us to directly observe the unwinding reaction of a DEAD box helicase on long stretches of double stranded RNA (dsRNA). Specifically, we could differentiate between the binding of DbpA to RNA in the absence of ATP and the formation of a Y shaped intermediate after its progression trough dsRNA in the presence of ATP. Recent studies have questioned the designation of DbpA, in particular, and DEAD-box proteins in general as RNA helicases. However, accumulated evidences and the results reported here suggest that these proteins are indeed helicases that resemble in many aspects the DNA helicases.

Keywords: RNA HELICASES, AFM, ATP

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VISUALIZING MOLECULAR PACKING ARRANGEMENTS ON PROTEIN CRYSTAL FACES BY AFM

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The objective is to determine the structure of the protein surface. Realizing this goal is difficult because the image observed is dependent upon many parameters. If both the sample and the tip consisted of rigid, uncharged atoms, one would have to consider only the topology of the atoms on the surface of the tip and the sample. However, the surface atoms may be displaced from their equilibrium positions by interactions with the tip. Additionally, various sample regions interact differently with the tip. Low resolution images may directly indicate the low resolution morphology of the scanned surface.
However, when features within individual molecules are observed, they result from small protrusions from the tip contacting the sample surface. It is difficult or impossible to measure tip features on the order of several angstroms. In a attempt to overcome these difficulties and obtain high resolution information, we strive to collect images in a manner that permits the 'rigid surfaces' approximation to be approximately valid. We then construct a molecular model of the surface and find the tip geometry that, when convoluted with the model, yields a theoretical image that best agrees with the experimental image. The plausibility of the model is judged by the agreement between the two images.
The model and tip are then adjusted to maximize this agreement. While obtaining a unique model of the surface from a single image is not possible, constraining the molecular model with known stereochemistry and analyzing multiple images limits the structures to be considered.

Keywords: AFM CRYSTAL GROWTH

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ATOMIC FORCE MICROSCOPY IN THE STUDY OF VIRUS PARTICLES, VIRUS CRYSTALS, AND VIRAL INFECTED CELLS

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Atomic Force Microscopy (AFM) is an effective technique for imaging virus particles within purified preparations, in crystalline form, and as they emerge from infected host cells. While the resolution of AFM does not approach that of X-ray crystallography, it can, in many cases, resolve capsomeres and other structural features on virion surfaces. Its investigative range in three dimensions serves to bridge the size interval of 1 nm to 1 μm lying between diffraction methods and light microscopy. We have used AFM to record a broad range of viruses ranging in diameter from 17 nm satellite plant viruses, to larger animal viruses such as herpes simplex (HSV) and mouse leukemic virus (MuLV) having diameters of about 100 nm, to even larger specimens such as irridoviruses and vaccinia. We present images that include particles arrayed on mica substrates (tobacco mosaic virus (TMV), cauliflower mosaic virus (CaMV), tipula iridescence virus (TIV), and HSV), crystals of viruses ((turnip yellow mosaic virus (TYMV), satellite tobacco mosaic virus (STMV), and brome mosaic virus (BMV)), and budding virions (MuLV from infected NIH 3T3 cells). We have also used AFM to study the structural transformation in budding virions of the MuLV retrovirus that occurs as a result of mutations, and to visualize the chemical and enzymatic dissection of HSV and vaccinia virus by detergents and proteases. From these studies, the architectural principles of very large viruses, which are not amenable to crystallography, and irregular or polymorphic viruses difficult to address with cryoelectron microscopy, can be delineated. Our experiences have shown that established techniques used in electron microscopy, such as immunolabeling with gold particles, and traditional histological and chemical treatments may be utilized as well with AFM.

Keywords: AFM VIRUS IMAGING

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HIGH-RESOLUTION ATOMIC FORCE MICROSCOPY IMAGED COMPLEX STRUCTURE OF MOLECULAR CHAPERONE Hsp70 AND Hsp40

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Molecular chaperone Heat shock protein 70 (Hsp70) pairs with a specific co-chaperone Heat shock protein 40 (Hsp40) to promote essential cellular functions such as protein folding, assembly, translocation *in vivo*. We have used atomic force microscopy (AFM) to image the protein interactions between molecular chaperone yeast Hsp70 Ssa1 lid domain (Lid) and yeast Hsp40 Sis1 C-terminal peptide-binding fragment (C-Sis1) at the single molecular level. The C-Sis1 protein molecules were clearly revealed under AFM and the AFM image of C-Sis1 is consistent well with the crystal structure of C-Sis1 dimer. Because the crystal structure of the protein complex between Hsp70 and Hsp40 is not available, we utilized AFM to study the Hsp70-Hsp40 interactions. The AFM images showed the protein complex formation of Hsp70 Ssa1 lid domain (Lid) and Hsp40 Sis1 C-terminal peptide-binding fragment (C-Sis1). The crystal structure of C-Sis1 dimer indicated a putative Hsp70-binding groove. The binding site between C-Sis1 and Lid was observed close to the vertex of groove of the C-sis1 dimer by AFM studies. The AFM images also indicated that Lid may dock into the putative Hsp70-binding groove of Hsp40 Sis1 peptide-binding fragment. The molar ratio of C-Sis1 dimer to Lid monomer in the complex is 1:1, which has been verified by the gel filtration chromatography. An 'Anchor and docking' model was postulated to illustrate the mechanisms by which Hsp70 interacts with Hsp40. This work proved that AFM is a valuable approach for studying protein-protein interactions at the single molecule level.

Keywords: MOLECULAR CHAPERONE AFM