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Quantitative non-isotopic nitrocellulose filter binding assays: Bacterial MnSOD-DNA interactions

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3078-Pos Board #B330

The Nucleocapsid Catalyzed Maturation of the Dimerization Initiation Site of HIV-1 is Modulated by pH-Dependent RNA Conformational Dynamics

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Abstract.
In the human immunodeficiency virus type 1 (HIV-1), the dimerization initiation site (DIS) has been identified as the RNA sequence primarily responsible for initiating the non-covalent linkage of two homologous strands of genomic RNA during viral assembly. The DIS loop contains an auto-complementary hexanucleotide sequence flanked by conserved 5' and 3' purines and can form a homodimer through a loop-loop kissing interaction. In a structural rearrangement catalyzed by HIV-1 nucleocapsid protein (NCp7) and suggested to be associated with maturation of the budded virus, DIS converts from the kissing homodimer to an extended duplex. In this study we have used heteronuclear multidimensional NMR spectroscopy to demonstrate that the DIS kissing dimer displays conformational dynamics that is specifically associated with protonation of the N1 base nitrogen of DIS loop residue A272 at close to neutral pH. In addition, we prove using a fluorescence spectroscopy functional assay that the rate of NCp7 catalyzed maturation of the DIS dimer is also pH dependent, with the more dynamic A272 protonated state associated with a faster rate of NCp7 action. The implications of these results, that suggest a role for RNA base protonation in modulating local structural stability and in promoting refolding from a kinetically stable intermediate conformation to an alternative thermodynamically more stable structure, will be discussed.

3079-Pos Board #B331

Assembly of the L3-L6-rRNA complex in B. stearothermophillus shows cooperativity.

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Ribosome assembly is a directed process involving the sequential binding of ribosomal proteins. The molecular mechanism of this process is not understood. However, any attempt at a detailed analysis of these interactions is possible only in the context of a smaller fragment of rRNA. The sarcin-ricin domain in the 23S rRNA is suitable for such a study. It contains the sarcin-ricin loop (srl) which is highly conserved, and a functionally important part of the ribosome. X-ray and biochemical studies show that the ribosomal proteins L3, L6 and L14 bind this region. The preparation of a 156 nt rRNA 4-helix junction (srl156) containing the srl and regions contacted by the three ribosomal proteins allows investigation into the assembly of the protein:rRNA complex.
The binding of ribosomal proteins L3 and L6 was characterised using filter-binding assays. L3 mainly recognises the junction of srl156. Once formed, the L3-srl156 complex is very stable. However complex formation requires a heating step and the presence of Mg2+, suggesting that reorganisation of the rRNA is needed for binding L3. L6 is seen in the crystal structures to contact mainly the 2730 and srl loops. L6 binding to the srl156, the srl156 with the srl deleted, and stem loops containing either the srl or the 2730 loop, was assayed over a wide range of salt concentrations. The results indicate that L6 primarily recognises the 2730 loop.
Qualitative gel-shift assays indicate cooperativity in the binding of L3 and L6 to srl156, though no contact is seen between the two proteins in the crystal structures. We are trying to quantitate the cooperative effect using equilibrium methods such as fluorescence. Our studies suggest that L3 may reorganise the junction such that L6 is able to contact both 2730 and srl loops

3080-Pos Board #B332

Water release accompanying specific versus nonspecific BamHI-DNA binding.

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It is generally conceded that hydration water plays an important role in DNA-protein sequence-specific recognition. A difference in the numbers of water molecules released in the binding of a protein to two different DNA sequences can be measured through the dependence of the relative binding constant on water activity (or, equivalently, on osmotic pressure). We showed previously that a nonspecific complex of the restriction nuclease EcoRI sequesters about 110 water molecules more than the complex with the specific recognition sequence. We are starting now to measure water release accompanying DNA binding reaction for another type II restriction endonuclease, BamHI. X-ray structures for both BamHI specific sequence and non-cognate sequence complexes are available (Newman M. *et al* (1995) Nature **368**, 660; Viadu H. and Aggarwal A.K. (2000) Molecular Cell **5**, 889). Unlike the specific sequence complex, the non-specific complex structure shows a gap between the BamHI and DNA major groove surfaces that is large enough to hold ~150 waters. We are applying osmotic stress technique to measure differences in number of water molecules retained in non-specific and specific BamHI-DNA complexes. Preliminary results show ~ 130 extra water molecules retained in the non-specific complex versus specific one presumably at the DNA-protein interface. Interestingly, the osmotic dependence of the absolute binding constant of the BamHI to its specific sequence revealed only about 20-25 waters released in the process of protein binding (Lynch T.W. and Sligar S.G. (2000) JBC **275**, 30561). Possible explanations for the differences in numbers of water molecules obtained by absolute and relative binding measurements are discussed.

3081-Pos Board #B333

Quantitative Non-Isotopic Nitrocellulose Filter Binding Assays: Bacterial MnSOD-DNA Interactions

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Nitrocellulose filter binding assays (NCFBAs) have been used for many years to quantitatively determine protein-nucleic acid affinities. While this technique is robust thermodynamically and fairly simple to perform, the requirement of radiolabeled nucleic acids has several major drawbacks. Some disadvantages are the short half-life of 32P, and over time the beta emissions cause fragmentation of the nucleic acids. Other drawbacks are the inherent safety concerns and cost of working with radioisotopes. We have modified standard NCFBAs by developing a quantitative nonisotopic method. We have replaced the radiolabeled DNA with biotin-labeled DNA. The biotin tag is detected by streptavidin-conjugated alkaline phosphatase. The alkaline phosphatase, in turn, catalyzes the degradation of 1,2-dioxetane, which produces photons as a by-product. Quantitation of the photon emissions is simplified by use of a cooled CCD camera. The binding affinity of bacterial manganese superoxide dismutase (MnSOD) to DNA has been quantitated by standard NCFBAs (Steinman, HM, Weinstein, L, Brenowitz, M. 1994. J. Biol. Chem. 269: 28629-34.). We have replicated that published binding study using the non-isotopic method that we have developed. We provide quantitative agreement between the isotopic and non-isotopic methods.

3082-Pos Board #B334

Time-resolved resonance Raman characterization of a neutral tryptophan radical in DNA photolyase and the effect of the substrate electric field on its lifetime

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DNA photolyase is a blue-light photoreceptor that repairs UV-induced cyclobutane pyrimidine dimers in DNA. Its active site contains a flavin adenine dinucleotide (FAD), which is fully reduced in the active form of the enzyme. The isolated enzyme contains the neutral radical semiquinone form of FAD (FADH*), which can be activated to its reduced form by photoinduced intraprotein electron transfer. The final result is fully reduced FAD and a neutral Trp radical (Trp*), which has a lifetime of several milliseconds. Trp radicals are not only of great interest in blue-light photoreceptors but have recently been cited in ribonucleotide reductase and monoclonal antibodies as well. We have successfully measured the resonance Raman spectrum of a Trp* intermediate in DNA photolyase, and this offers a new way to study Trp radical intermediates in electron-transfer processes and catalytic mechanisms, and their interactions with the protein environment. Furthermore, we have determined that the lifetime of the Trp* increases when DNA photolyase binds to UV-damaged DNA in a pH-dependent manner. This effect is most likely brought about by the electric field of the substrate. We will report on our progress of the characterization of the intermediate Trp* by resonance Raman spectroscopy and the effect of the substrate electric field on its lifetime in DNA photolyase.

Virus Structure & Assembly

3083-Pos Board #B335

Measurements of pressurized DNA in phage capsids

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A dsDNA bacteriophage packs its genome into a tiny capsid, creating an internal pressure as high as 20 atm that is thought to power the ejection of its genome into cells. We measure the pressure by balancing it with an external osmotic pressure, causing an in vitro partial ejection of the packaged DNA. Using various strains of lambda and phi29 bacteriophage, we examine the