Protein-Ligand Interactions II

2335-Pos Board B105

Cloning of a Novel Centrin Target Peptide

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The origin of many disease states has been linked to genetic mutation, defects in gene expression, nuclear excision repair and ribosome biogenesis. Centrin, a calcium binding protein, has recently been found to regulate some of these processes along other target proteins within the nucleus. One target, Krr1, contains a K homology (KH) domain; which has been identified as a nucleic acid recognition motif, required for proper processing of pre-rRNA, for synthesis of 18S rRNA, and for the assembly of the 40S subunit. Our initial findings have identified a putative centrin binding site located within the KH domain of Krr1 within the Homo sapiens (Hs) centrin 2 (Hscen2-HsKrr1 complex) using bioinformatics tools. In this study, the KH domain (192 bp) was amplified by PCR and then ligated to the expression vector pET100 which adds a His tag to the peptide. Colony PCR was performed to identify the E. coli colonies that have been transformed effectively with the desired recombinant. The KH domain was then expressed in E. coli cells. To identify the presence of this peptide in the bacteria, an SDS-PAGE was performed. The overexpressed KH domain peptide will be purified and used for interaction studies with Hscen2.

2336-Pos Board B106

Reduced Nicotinamide Adenine Dinucleotide Phosphate (NADPH) Blocks Transfer of [2Fe-2S] Clusters from MitoNEET to an Apo-Acceptor Protein

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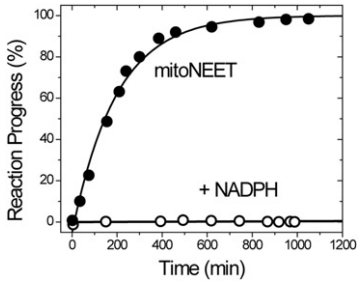
MitoNEET is the founding member of the recently discovered CDGSH family of [2Fe-2S] proteins. Its structure contains a unique homodimeric fold with each monomer containing one [2Fe-2S] cluster (1). MitoNEET is a target of the thiazolidinedione (TZD) class of anti-diabetes drugs (2). Recently, it was reported that the [2Fe-2S] cluster in mitoNEET is destabilized upon binding of NADPH/NADP+ (3). As mitoNEET is capable of transferring its [2Fe-2S] clusters to an apo-acceptor protein (4) and an obvious question to ask is whether NADPH binding accelerates or abrogates cluster transfer. We show that NADPH blocks transfer of mitoNEET's [2Fe-2S] cluster to an apo-acceptor protein (see Figure). This suggests a likely functional role for the mitoNEET:NADPH/NADP+ interaction.



(1) Paddock ML et al. (2007) *PNAS* 104 14342-7
(2) Colca JR et al. (2004) *Am J Physiol Endocrinol Metab* 286 E252-60
(3) Zhou et al. (2010) Biochemistry 49 9604-12
(4) Zuris JA et al. (2011) *PNAS* 108 13047-52

*Supported by the Heme and Blood Proteins Training Grant 5T32DK007233-34

2337-Pos Board B107

Mapping the Inositol Hexakisphosphate Binding Sites on the Human Fibroblast Growth Factor

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An investigation of the characteristics of the interaction between inositol hexakisphosphate (IP_6) and the human acidic fibroblast growth factor (hFGF-1) was performed. Results of the Isothermal titration calorimetry experiments suggest that binds IP_6 to hFGF-1 with a high affinity. Interestingly, differential scanning calorimetry data show a significant reduction in the melting temperature (T_m) suggesting a reduction in the stability of the protein (hFGF-1) upon binding to IP_6. This is further confirmed by the increased rate of proteolytic digestion of hFGF-1 in the presence of IP_6 . Circular dichroism and intrinsic fluorescence spectroscopy data indicate that the ligand (IP_6) does not induce major secondary structural changes in the protein. ^1H ^{15}N chemical shift data reveal that IP_6 binds in the heparin binding pocket in hFGF-1. The significance of binding of IP_6 to hFGF-1 and the prospects of designing novel therapeutic principles against hFGF-1 induced pathogenesis will be discussed.

2338-Pos Board B108

A Two-Photon Absorption Probe Mimicking NADPH that Inhibits the Nitric Oxyde Synthase

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Synthesis of nitric oxide is performed by NO-synthase. The catalysis is initiated by the combined transfer of electrons/proton from NADPH to the flavin FAD. We have previously characterized one photoactive compound (named Nanotrigger, NT1) [1,2] allowing to trigger and synchronize NOS activity upon light illumination. Here, we described a new compound (NS1) with two-photon absorption properties suitable to assess the binding to the NOS protein. This family of compounds combines a docking moiety (NADPH analog) and a chromophore moiety responsive for light illumination. NS1 was characterized in DMSO by an absorption maximum at 460nm and was found to be fluorescent with an emission peak centered at 740nm upon one-photon excitation. However, the fluorescence emission was strongly sensitive to solvent polarity as evidenced by the significant decrease in the emission intensity in polar solvents. The same behaviour was observed under two-photon excitation (exc, 940nm). One- and two-photon fluorescence approaches were used to assess the binding of NS1 to the neuronal NOS. Under one-photon excitation, the emission properties of the complex were rather difficult to interpret due to the overlapping between the intrinsic fluorescence of nNOS and NS1 fluorescence. As the two-photon fluorescence of nNOS protein is very weak, the complex formation was measured by monitoring the two-photon fluorescence recovery of NS1 upon binding to nNOS. The calculated Kd value was found to be consistent with the value characterizing NADPH binding. However, competition experiments suggest that the competition between NS1 and NADPH is more complex than expected from a one-binding site model. Our results suggest that NS1 represents a promising compound for cellular applications in two-photon fluorescence imaging.

[1] Beaumont et al., J. Am. Chem. Soc. 2007, 129, 2178-2186
[2] Beaumont et al., ChemBioChem 2009, 10, 690-701

2339-Pos Board B109

Kinetics of Ligand Receptor Interaction Reveals the Mode of Binding in Cyclic Nucleotide-Activated Proteins

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Structural biology has provided a wealth of information about the three-dimensional organization of proteins. The next ultimate goal is to watch proteins in action, i.e. to study their dynamic nature. A key question in the field of ligand-activated proteins is the mode of ligand binding - does the ligand induce a conformational change in the protein - induced fit - or does the protein preexist as an ensemble of conformations and the ligand selects the most complementary one - conformational selection? The particular mode of binding determines the 'personality' of the protein: is it highly dynamic or rather static? Finally, it is important to know how long a protein gets activated by a particular ligand. Therefore, the residence time of a ligand at the binding site is a significant subject.

We study here the kinetics of cAMP binding to a cyclic nucleotide-binding domain (CNBD) in its isolated monomeric form. CNBDs are ancient, highly conserved domains that control a multitude of different proteins: Among them are transcription factors (CAP), nucleotide exchange factors (EPAC), protein kinases (PKA/PKG), as well as ion channels (CNG/HNC). As a model system we have chosen a cyclic nucleotide-gated channel from Mesorhizobium loti (MlCNG). The rate constants of ligand binding were determined using rapid mixing techniques in conjunction with a solvatochromic, fluorescent ligand (8 NBD cAMP). The consequence of a mutation on the rate constants reveals the mode of ligand binding. Comparing experimental rate constants to those calculated by a computational model provides excellent agreement. Moreover, by studying theoretical ligands, i.e. neutralizing or inverting charges, we gained deeper insight into the binding process.