Title

Applications of Genetically Encoded Unnatural Amino Acids for Protein Structure Mapping by Site-Directed Spin Labeling

Journal

Biophysical Journal, 100(3)

ISSN

0006-3495

Authors

Fleissner, Mark R
Bridges, Michael D
Kálai, Tamás
et al.

Publication Date

2011-02-01

DOI

10.1016/j.bpj.2010.12.478

2-oxyoglutarate-Fe(II) dioxygenase protein superfamily. Unlike the prolyl hydroxylase PHD which hydroxylates proline residues in the hypoxia-inducible factor HIF, Ofd1 utilizes its N-terminal dioxygenase domain to control the interaction between itself and its inhibitor Nro1: in hypoxia, Nro1 binds to Ofd1 and this inhibits its C-terminal degradation domain (CTDD) function on Sre1N destabilization. As shown previously, $Ofd1_{CTDD}$ by itself is sufficient to promote Sre1N degradation and there's direct interaction between Nro1 and $Ofd1_{CTDD}$. However, the molecular mechanism by which $Ofd1_{CTDD}$ accelerates Sre1N degradation remains unclear. Here we report the crystal structure of $Ofd1_{CTDD}$ at 2.0 Å resolution. Interestingly, $Ofd1_{CTDD}$ has a double-stranded beta helix (DBSH) fold like that of the 2-oxyoglutarate-Fe (II) dioxygenase protein superfamily but lacks the iron-binding motif characteristic of the superfamily. This structure may help elucidate how $Ofd1_{CTDD}$ promotes Sre1N turnover and how Ofd1-Nro1 as a whole functions as an oxygen-sensor.

263-Pos Board B63

Structure of the Sec13/31 & Sec23 COPII coat cage

NIlakshee Bhattacharya, Jason O'Donnell, Abbas Razvi, William E. Balch, Scott M. Stagg.

COPII coated vesicles are responsible for packaging and transporting newly synthesized proteins from the endoplasmic reticulum to the Golgi apparatus. The COPII coat consists of Sec13/31, Sec23/24, and Sar1. Mutation in these coat protein cause medical conditions like Anderson disease, chylomicron retention disease and cranio-lenticulo-sutural dysplasia, which highlights the biological relevance of the coat proteins. Previously we solved two different COPII structures (Stagg et. Al., Nature 2006 and Stagg et. Al., Cell 2008) that suggest that the hinge region formed by the four heterotetramer can direct cage expansion to accommodate cargo of various sizes. Recently a tubular structure of Sec 13/31 solved where the tubules were formed by the concatenation of individual sec13/31 cage (O'Donell et. Al, J. STruc. Biol.). Earlier, we hypothesized that the distribution of Sec23/24 dictates the geometry of the COPII coat. We now show that Sec23 by itself influences the outer geometry of the cage. We have reconstructed a structure of a COPII coat cage assembled from Sec13/31 and Sec23. The assemblies form at least two geometries, and the most common size is 600 Å, similar to what has been observed for Sec13/31. We will discuss how the orientation of Sec23 may dictate cage geometry and orient Sar1 to participate in the fission of COPII coated vesicles in the cell.

264-Pos Board B64

Crystallization of Glutaraldehyde Cross-Linked Prothrombinase Complex in its Active Form

Cheryl L. Law, Rima Chattopadhyay, Rinku Majumder, Barry R. Lentz.

Activation of prothrombin to thrombin is catalyzed by ''prothrombinase'' complex, consisting of factor Xa and its cofactor factor Va bound to phosphatidylserine (PS)-containing membrane. While partial crystal structures of Xa and Va exist, attempts to crystallize XaVa complex failed. Recent studies shows that both Xa and Va2, an active isoforms of Va binding tightly to PS-membrane, binds soluble dicaproyl-phosphatidylserine (C6PS). Also, C6PS-bound form of Va2 binds with high affinity (Kd ~ 1 nM) to Xa forming a fully active prothrombinase complex in solution. Preliminary studies show this soluble complex, when cross-linked with glutaraldehyde, remains intact after removing C6PS using size-exclusion chromatography. Depending on conditions, up to 92% of initial activity is restored after adding back C6PS. To determine optimal formation conditions of an active cross-linked XaVa complex for use in crystallization trials, we used an algorithm varying Xa/Va and glutaraldehyde concentration, incubation time, and temperature. XaVa activity was tested after four critical stages (complex formation, cross-linking with glutaraldehyde, removing C6PS, reactivation with C6PS) by a chromogenic assay, and the yield of cross-linked complex was determined by using quantitative Shodex size-exclusion chromatography. While preliminary experiments were performed using plasma-derived human Va2, greater quantities of protein are needed than can be produced by this method. Since crystallization is more successful with cloned recombinant proteins, our lab works with multiple labs to establish procedures for expressing large quantities of recombinant human Va2 (rHVa2) in Baby Hamster Kidney cells (BHK). With higher yields of Va2, we are proceeding with trials to optimize conditions for cross-linked active prothrombinase complex. If these are successful, we will continue with crystallization trials, enabling us for the first time to determine the structure of XaVa complex in its active form. Supported by grant HL072827 and a training supplement thereto.

265-Pos Board B65

Crystal Structures of NAD(P)(H) Sensing NmrA-Like Proteins from *Dictyostelium Discoideum*

Min-Kyu Kim, **Hyung-Soon Yim**, Sa-Ouk Kang.

NAD(P)(H) functions as central coenzyme in catabolic and anabolic reaction. And recently, many results show that NAD(P)(H) is also involved in signaling pathways that regulate fundamental processes of cellular functions. NmrA in *Aspergillus nidulans* is known to discriminate between oxidized and reduced dinucleotides and suggested as redox sensor. There are several NmrA-like protein coding genes in *Dictyostelium discoideum* and they show developmental stage-dependent expression levels and especially DDB_G0291732 gene exhibits different expression pattern from the other NmrA-like protein coding genes. To study relation between structure and function, the crystal structures of DDB_G0291732 and DDB_G0286605 were determined. Crystal structures of DDB_G0291732 and DDB_G0286605 adopt the features of SDR-type NmrA-like proteins. DDB_G0291732 and DDB_G0286605 contain conserved N-terminal dinucleotide binding Rossmann fold with a central β sheet and the C-domain, containing a hydrophobic pocket connected to the N-domain. Structural comparisons with known 3D structures reveal that DDB_G0291732 and DDB_G0286605 are mostly matched with HSCARG; human NADP(H) sensor protein, NmrA; negative transcriptional regulator involved in nitrogen metabolite repression of *Aspergillus nidulans* and QOR2; new-type quinone oxidoreductase considered to function as transcriptional regulator of *E. coli*. No dehydrogenase activity could be prospected with DDB_G0291732 and DDB_G0286605 because the catalytic Tyr residue in the active site triad of Ser-Tyr-Lys is replaced by His and Asp in DDB_G0291732 and DDB_G0286605, respectively. Cofactor binding experiments reveal that DDB_G0291732 does not interact with pyridine dinucleotides and DDB_G0286605 has high affinity with NADP(H) but not with NAD(H). The mRNA expression levels of DDB_G0291732 and DDB_G0286605 genes after dehydroepiandrosterone (DHEA) or H_2O_2 treatment are monitored to examine how these proteins respond to redox states in *D. discoideum*.

266-Pos Board B66

Applications of Genetically Encoded Unnatural Amino Acids for Protein Structure Mapping by Site-Directed Spin Labeling

Mark R. Fleissner, Michael D. Bridges, Tamás Kálai, Jun Y. Axup, Douglas D. Young, Kálmán Hideg, Peter G. Schultz, Wayne L. Hubbell.

Site-directed spin labeling (SDSL) is an important method for obtaining structural information on proteins that are refractory to other techniques. In this method, interspin distances are used as structural constraints for protein structure mapping, and here we present two applications of genetically encoded unnatural amino acids for such studies. One application is orthogonal labeling, in which an unnatural amino acid bearing a unique functional group is used to specifically introduce spin labels into proteins that are not amenable to the traditional, thiol-based SDSL method. Recently, a strategy based on *p*-acetyl-L-phenylalanine was reported [*PNAS* 2009 106(51):21637], in which the ketone was used to generate a ketoxime-linked spin label (K1). Here, we present an analogous strategy based on *p*-azido-L-phenylalanine, which can be specifically modified using "click" chemistry by a cyclooctyne-nitroxide reagent to generate a triazole-linked nitroxide side chain (C1). In contrast to the previous orthogonal labeling strategy, the azide-cyclooctyne reaction proceeds uncatalyzed at pH 7, and spectra of C1 mutants of T4 lysozyme (T4L) reflect a more ordered nitroxide motion than the analogous K1 mutant. A second application involves the Cu(II) binding unnatural amino acid (2,2'-bipyridin-5-yl)alanine (BpyAla), which enhances the longitudinal relaxation rate ($1/T_1$) of a nearby nitroxide when copper is bound, thereby allowing average interspin distances to be measured at ambient temperature using saturation recovery EPR. In contrast to other methods for paramagnetic metal introduction, BpyAla can be introduced via a single mutation into any protein topology. Studies of T4L mutants containing BpyAla and a disulfide-linked nitroxide indicate that interspin distances of up to ~30Å are accessible to T_1 based EPR methods compared to ~20Å for traditional T_2 based methods. Both applications are expected to enhance distance mapping capability by EPR spectroscopy.

267-Pos Board B67

Structural Characterization and Kinetic Analysis of a Bacterial-Type 6-Pyruvoyl Tetrahydropterin Synthase from *E. Coli*

Kyung Hye Seo, ning ning Zhang, cong Chen, Young Shik Park, **Kon H. Lee**.

6-pyruvoyl tetrahydropterin synthase (PTPS) is a second enzyme in the biosynthesis of terahydrobiopterin (BH4). It catalyzes generally the conversion of dihydroneopterin triphosphate (H2NTP) to 6-pyruvoyl tertrahydropterin