2278-Pos Board B248

Reaction Mechanisms of Editing (Proofreading) by Leucyl-tRNA Synthetase Revealed by QM/MM Molecular Dynamics Simulations

Masaru Tateno[1], Yohsuke Hagiwara[1], Osamu Nureki[2].
[1]University of Tsukuba, Tsukuba Science City, Japan, [2]University of Tokyo, Tokyo, Japan.

Aminoacyl-tRNA synthetases (aaRS's) play a critical role in decoding genetic information located on genome DNA sequence, through catalyzing attachment of their cognate amino acid to 3′-end of the specific tRNA. The fidelity of translation is assured by their strict discrimination of the cognate amino acids from non-cognate ones. However, in the case of valine, isoleucine, and leucine systems, it is difficult for the cognate enzymes to discriminate their specific amino acid; thus, the synthetases produce mis-aminoacylated tRNAs, such as Ile-tRNA^{Leu}. However, the mis-products are hydrolyzed through the ''editing'' reaction by the aaRS's.
In this study, we performed molecular docking simulations of the LeuRS•tRNA^{Leu} complex and an incognate amino acid (Val), using a novel algorithm referred to as the Fully Solvated Dynamical Docking (FSDD) scheme which is developed by our group (see the presentation by Hagiwara, Y., et al.). Thus, we have successfully identified ordered water molecules in its active site, and also, one of such waters has been found to be located at the appropriate position as the nucleophile.
Then, for the modeled structure of the LeuRS•Val-tRNA^{Leu} complex, we performed QM/MM calculations using our new interface program connecting QM (gamess) and MM (amber) engines, which has also been developed by us. Thereby, it has been found that LUMO is located on the reaction point on the substrate, suggesting that the water identified actually attacks the orbital as the nucleophile. In fact, as the distance between the substrate and the water (O atom) is decreased, the orbital energies of 2*p* electrons of the oxygen atom is elevated toward the energy level of HOMO. In the presentation, we will show recent results of our QM/MM-MD simulations performed to elucidate detailed reaction mechanisms of the editing.

2279-Pos Board B249

Redox activity and H_2 production upon glycerol fermentation in *Escherichia coli:* are hydrogenases reversible?

Karen Trchounian, Armen Trchounian.
Yerevan State University, Yerevan, Armenia.

The molecular hydrogen (H_2) can be produced through bacterial hydrogenases catalyzing the reaction of $2H^+ + 2e^- \rightarrow H_2$. Under fermentation of glucose *Escherichia coli* hydrogenases (Hyd) 3 and 4 are responsible for H_2 production depending on pH [1] and formate [2] and Hyd-1 and Hyd-2 operate as H_2 uptake hydrogenases. Hyd-3 was shown recently can work in reverse mode [3].
Redox activity and H_2 production are studied under glycerol fermentation by *E. coli* at acidic [4] and slightly alkaline pH. Redox potential was shown to decrease upon glycerol fermentation at pH 7.5. The H_2 production rate was ~5-fold less in *hybC* (Hyd-2) than that in *hyaB* (Hyd-1) mutant but it was lower than that in wild type strain or *hycE* (Hyd-3) and *hyfG* (Hyd-4) mutants. Interestingly, 0.5 mM *N,N′*-dicyclohexylcarbodiimide (DCCD) noticeably inhibited H_2 production in wild type and mutants used; at the same time DCCD inhibition of H_2 production rate was less and H_2 production was ~14 times higher in *hyaB* than in *hybC* mutant. The results point out that Hyd-2 plays a main role in H_2 production under glycerol fermentation; a participation of Hyd-1 is not ruled out although Hyd-3 and Hyd-4 don't operate under these conditions.
E. coli Hyd-2 might be concluded to work in reverse mode similar to Hyd-3 [3] but in different conditions.

References
[1] Bagramyan K., Mnatsakanyan N., Poladian A., Vassilian A., Trchounian A.(2002) FEBS Lett. **516**,172-178.
[2] Mnatsakanyan H., Bagramyan K., Trchounian A. (2004) Cell Biochem. Biophys. **41**, 357-366.
[3] Maeda T., Sanchez-Torres V., Wood T.K. (2007) Appl. Microbiol. Biotechnol. **76** 1035-1042.
[4] Dharmadi Y., Murarka A., Gonzalez R. (2006) Biotechnol. Bioeng. **94**, 821-829.

Protein-Ligand Interactions I

2280-Pos Board B250

Localizing Fatty Acid Binding Sites on Human Serum Albumin by 2D-NMR

Eileen Krenzel, Zhongjing Chen, James A. Hamilton.
Boston University, Boston, MA, USA.

The most abundant protein in plasma, human serum albumin (HSA), is the principal carrier for endogenous lipophilic compounds and lipophilic drugs. Non-esterified long chain fatty acids (FA) are its primary physiological ligand, with multiple binding sites of varying affinities. Detailed structural understandings of HSA-ligand interactions are vital during the development of drugs. HSA ligand interactions have been studied by various biophysical methods: x-ray crystallography, fluorescence spectroscopy, and nuclear magnetic resonance (NMR). Numerous crystal structures of HSA, with and without drugs and/or FAs, have been published. Compared to crystallography, our solution state NMR studies are focused on the more physiologically relevant state. Previously, we identified three high affinity and several low affinity FA binding sites on HSA by 1D-NMR at low resolution. Now, we are correlating crystallographic data with high-resolution 2D-NMR spectra of 13C-methyl-labeled oleic acid (OA). Our 2D-HSQC spectra show the 13C-methyl group of OA bound to HSA. The unique microenvironments that each methyl group experiences in the binding pockets gives rise to different NMR signals of varying intensities. These correspond to the different affinities that HSA has for OA, and our 2D-NMR spectra clearly differentiate the three highest affinity-binding sites from six lower affinity-binding sites. We have identified Sudlow's Drug Sites by utilizing the known FA competitors and Sudlow's site binders: warfarin and diazepam. By expanding this drug competition strategy with ibuprofen and diflunisal, we have identified additional FA binding sites. The highest affinity FA binding sites do not have known competitors and these sites are identified with the use of site-directed mutagenesis of HSA. In summary, we are reporting an approach for studying the interactions of FAs with HSA and FA-competitive drugs in a site-specific manner through the use of state-of-the-art 2D-NMR techniques.

2281-Pos Board B251

Calcium-Dependent Interactions of Calmodulin with Calcineurin: Evidence for Domain-Reversal in Target Recogition

Susan E. O'Donnell, Madeline Shea.
University of Iowa, Iowa City, IA, USA.

Cardiac hypertrophy results from stress on cardiomyocytes, which contributes to heart failure. Constitutive activation of calcineurin (CaN), a dimeric Ser/Thr phosphatase, induces hypertrophic responses. Ca^{2+}-CaM is essential for CaN activation; however, the mechanism of activation is not fully understood. Recent reports have raised new questions regarding the stoichiometry of the CaM:CaNp complex, *in vitro* and *in vivo*. A recent structure showed CaM forms a novel 2:2 complex with the CaM-binding domain of CaN (CaNp, 2R28.pdb).
Hydrodynamic methods were used to determine the stoichiometry of the complex formed between Ca^{2+}-saturated *Paramecium* CaM_{1-148} (PCaM) and CaNp. Stokes radius (R_s) and sedimentation coefficient (s) values were consistent with a 1:1 complex. At higher protein concentration, ^{15}N T_2 relaxation rates of $(Ca^{2+})_4$-^{15}N-$PCaM_{1-148}$ in the presence of CaNp or melittin were compared. Results support a model in which CaM associates with CaN with a 1:1 stoichiometry. Under the same solution conditions, $(Ca^{2+})_4$-$PCaM_{1-148}$ has a very high affinity for CaNp (estimated K_d of 1 pM).
The response of individual PCaM residues to CaNp binding was studied. ^{15}N-HSQC spectra of ^{15}N-$(Ca^{2+})_4$-$PCaM_{1-148}$ showed that the addition of CaNp caused dramatic peak shifts. Backbone assignments for ^{13}C,^{15}N-$(Ca^{2+})_4$-$PCaM_{1-148}$ in the presence of CaNp were determined with standard 3D NMR experiments. This showed that residues from *both* domains of CaM experience conformational changes when bound to CaNp; however, the N-domain was perturbed to a greater extent. This is unusual when compared to all 17 known compact CaM-target structures, where the C-domain of CaM preferentially serves as a target-docking site. Support by AHA & UI CBB Predoctoral Fellowships to SEO, NIH R01 GM57001 to MAS.

2282-Pos Board B252

Structural Basis for Phosphate Stabilization of the Uniquely Coordinated 2Fe-2S Cluster of the Outer Mitochondrial Membrane Protein MitoNEET.*

Christina Homer[1], David Yee[1], Herbert L. Axelrod[2], Aina E. Cohen[2], Edward C. Abresch[1], Charlene Chang[1], Rachel Nechushtai[3], Patricia A. Jennings[4], Mark L. Paddock[1].
[1]Department of Physics, UCSD, La Jolla, CA, USA, [2]Stanford Synchrotron Radiation Laboratory, Menlo Park, CA, USA, [3]Department of Plant and Environmental Sciences4, The Wolfson Centre for Applied Structural Biology, The Hebrew University of Jerusalem, Givat Ram, Israel, [4]Department of Chemistry and Biochemistry, UCSD, La Jolla, CA, USA.

MitoNEET is the first outer mitochondrial membrane protein shown to contain a pH labile redox active 2Fe-2S cluster (1). Additionally, we found that phosphate at physiological concentrations (10 mM) stabilized the cluster from release by ~10-fold over a broad pH range from $5.0 \leq pH \leq 7.5$. The structure