systems, thus the inactive dimer conformation may be the keypoint in determining signaling specifity.

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Keywords: GTP-binding proteins, biophysical analysis, SAXS

FA1-MS06-P05

Crystal Structure of Protein-Glutaminase and Its Pro-Structures Similar to Enzyme-Substrate Complex. Bunzo Mikami[a], Ryota Hashizume[a] Kimihiko Mizutani[a], Nobuyuki Takahashi[a], Hiroyuki Matsubara[b], Akiko Matsunaga[b], Shotaro Yamaguchi[b]. [a]*Laboratory of Applied Structural Science, Graduate School of Agriculture, Kyoto University, Japan.* [b]*Gifu R&D Center, Amano Enzyme Inc.Japan.*
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Protein-glutaminase, which converts glutamine residues in protein or peptide to glutamic acid residues, is expected to see wide use as a new food processing enzyme. Deamidation of proteins can improve their solubility, emulsifying activity, forming activity, and other functional properties by increasing the number of negative charges. The crystal structure of mature protein-glutaminase with 185 amino acid residues was determined by the single-wavelength anomalous dispersion (SAD) method and refined at 1.15 Å. The overall structure, which consists of four helices and two antiparallel □-sheets with four and two strands, has a weak homology with the core domain of human tissue transglutaminase 2. The catalytic triad, Cys-His-Asp conserved in transglutaminases was also found in this enzyme. The important differences in the catalytic region from transglutaminase could be explained by their reaction specificity. The enzyme has large hydrophobic surface in order to interact with protein substrate. We also determined the structures of recombinant pro-enzyme and its mutant with 299 amino acid residues. It is remarkable that a short loop around Ala 47 in the pro-region covers and interacts with the active site of the mature region. Crystal structures of A47Q mutant showed that the side chain of Gln 47 interacts with the catalytic residue, Cys 156, forming a covalent bond between Cys156/Sγ and Gln47/Cδ depending on the pH of the crystallization medium. The structure of A47Q mutant provides insights into the catalytic mechanism of the enzyme which forms a covalent S-acyl intermediate before release of ammonia.

Keywords: protein-glutaminase, pro-enzyme, enzyme mechanisms

FA1-MS06-P06

How chromosomes interact with microtubules. Andrea Musacchio. *Department of Experimental Oncology, European Institute of Oncology, Milan, Italy*
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To be parted into two equal complements during mitosis, the sister chromatids need to establish secure connections with the mitotic spindle, a microtubule- and motor-based structure [1]. The point of attachment of chromosomes to spindle microtubules is named the kinetochore. This protein assembly contains up to 100 or more proteins. Our current challenge is to understand the molecular mechanism of the interaction of kinetochores with microtubules. A 10-protein assembly known as the KMN network is responsible for this interaction. We have combined different approached to gain a structural and functional understanding of the role of the KMN network in microtubule attachment. Furthermore, we are investigating the mechanisms through which the KMN network regulates feedback control mechanisms that are responsible for the fidelity of the chromosome segregation process, namely the spindle assembly checkpoint [2].

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Keywords: cell cycle proteins, chromosome dynamics, biological macromolecules

FA1-MS06-P07

An Archaeal Dim2 Homolog Forms a Ternary Complex with a/eIF2α and 16S rRNA. Koji Nagata[a], Min Ze Jia[a,b], Shoichiro Horita[a], Masaru Tanokura[a]. [a]*Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan.* [b]*Institute of Biophysics, Chinese Academy of Sciences, China.*
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Dim2p is a eukaryal small ribosomal subunit RNA processing factor required for the maturation of 18S rRNA. We have shown that an archaeal homolog of Dim2p, aDim2p, forms a ternary complex with the archaeal homolog of eIF2α, a/eIF2α, and the RNA fragment that possesses the 3'-end sequence of 16S rRNA both in solution and in crystal [1]. The 2.8-Å crystal structure of the ternary complex reveals that two KH domains of the aDim2p, KH-1 and -2, are involved in binding the anti-Shine-Dalgarno (SD) core sequence (CCUCC-3') and a highly conserved adjacent sequence (5'-GGAUCA), respectively, of the target rRNA fragment. The surface plasmon resonance results show that the interaction of the aDim2p with the target rRNA fragment is very strong with a dissociation constant of 9.8×10^{-10} M and that the aDim2p has a strong nucleotide sequence preference for the 3'-end sequence of 16S rRNA. On the other hand, the aDim2p interacts with the isolated α subunit and the intact αβγ complex of the a/eIF2, irrespective of the RNA binding. These results suggest that the aDim2p is a possible archaeal pre-rRNA processing factor recognizing the 3'-end sequence (5'-GAUCACCUCC-3') of 16S rRNA, and may have multiple biological roles *in vivo* by interacting with other proteins such as the a/eIF2 and the aRio2p.

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Keywords: archaeal Dim2p, archaeal eIF2, rRNA processing

FA1-MS06-P08

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