PRION 2016 TOKYO

In Conjunction with

Asian Pacific Prion Symposium 2016

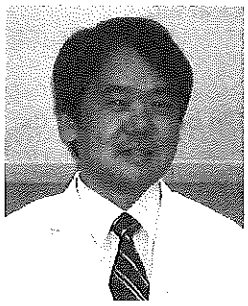
Program & Abstracts

DATE: **May 10 (Tue.) - 13 (Fri.), 2016**

VENUE: **Hitotsubashi Hall,**
National Center of Sciences Building

Welcome Message

Dear Colleagues,



On behalf of the Organizing Committee, I am pleased to announce that the Asian Pacific Prion Symposium (APPS) 2016 is going to be held in conjunction with PRION 2016 on May 10-13 in Tokyo, the capital city of Japan. This is the first historical event that almost all researchers in the prion-related fields with different disciplines in Asian Pacific and European areas gather in a hall and discuss on the same table.

Archaeological evidence indicates that the site of Tokyo was inhabited by Stone Age tribes. The present city was founded in the 12th cent. as the village of Edo. Landmarks include the Hie Shrine; the temples of Sengakuji, Gokokuji, and Sensoji; and the Korakuen, a 17th-century landscape garden. The Sky Tree, the highest (2,080 ft/634 m) self-supported structure of its type in the world, was completed in 2012.

The APPS 2016 in conjunction with PRION 2016 deals with a broad range of prion researches from basic to clinical aspects of prion diseases in multiple species. Especially here we have raised 'Overcoming Prion Diseases' as the main theme.

I sincerely hope everyone enjoy Tokyo, one of the most important cities as the corporate and communications hub for the east pacific rim as well as the APPS 2016 in conjunction with PRION 2016 providing a great opportunity of discussions that serve a trigger for the development of novel therapeutics for diseases caused by prion- and prion-like mechanisms.

APPS 2016 President

Kazuo Kuwata, M.D., Ph.D.

United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University

IL-26

Logical design of a therapeutic agent for prion diseases

Kazuo Kuwata

United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University, Japan

We have developed a logical design system targeting the prion diseases. First, we identified the susceptibe region in PrPC structure using NMR relaxation experiments, and various NMR experiments under the in vitro conversion conditions. Second, we developed a drug design software, 'NAGARA' which includes docking simulation, molecular dynamics simulation and quantum chemical calculation. Using NAGARA, we were able to discover the novel anti-prion lead compound and optimize it. Third, for the first time in academia, we installed various organic synthesis system including automatic robotic combinatorial synthesis system, organic synthesis system based on GMP standards, and pharmaceutical preparation system for injection based on GMP standards. Fourth, we developed the in vivo treatment examination system for prion diseases considering administration methos as well as pharmaceutical kinetics measurements for various animals including non-human primates. Fifth, we organised the Japanese Consortium of Prion Diseases (JACOP, President: Dr. Mizusawa) for clinical trial of the candidtae compounds for prion diseases.

Initial candidate would be a medical chaperone (MC). MC is the anti-prion compound optimized from the lead compound, GN8. It binds to the hot-spot of PrPC and strongly inhibited the conformational conversion reaction from PrPC to PrPSC. We invetstingated various in vivo effects due to the administration of this compound.

Curriculum Vitae

Kazuo Kuwata

Research Careers and Experience

1984.4-1993.3: Research Assistant, School of Medicine, Gifu University
Research theme: Kinetics of molecular aging of bovine serum albumin NMR relaxation time measurements of water in biological system

1992.4-1994.3: Postdoctoral Research Scientist, Faculty of Science, Department of Chemistry & Biochemistry, University of California Santa Cruz
Research theme: off-resonance rotating frame NMR relaxation time measurements
Newly developed off-resonance ROESY pulse sequence

1993.4-2004.3: Associate Professor, School of Medicine, Gifu University
Research theme: Molecular dynamics simulation of protein-water system
NMR studies on protein structure and dynamics

1999.4-2000.3: Associate Professor, Institute for Protein Research, Osaka University
Research theme: NMR structure determination of beta-lactoglobulin
Folding kinetics of beta-lactoglobulin (Continuous Flow & Pulse Label)

2004.4-present: Professor & Director, Center for Emerging Infectious Diseases, Gifu University
Research theme: Structural biological study on the prion's propagation mechanism
Rational design of anti-prion drug

Research Topics: Protein Dynamics, Folding, Stability and Prion propagation

O-01

Folding and misfolding pathways of prion protein

Ryo P. Honda[1], Kei-ich Yamaguchi[2], Kazuo Kuwata[2]

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The mechanism by which a random-coil polypeptide folds into the native structure may be critical for the regulation of misfolding diseases. However, how the folding pathway is related to the misfolding pathway remains unclear. Our recent study demonstrated that the native folding pathway of prion protein (PrP) involves at least four independent species, including native state (N), unfolded state (U), and two types of partially folded states (A and I) (Honda et al., Structure, 2015). Interestingly, one of the partially folded states (A state) readily formed a misfolded aggregate whose rates were strongly correlated with the initial population of the A state (Honda et al., J Biol Chem, 2014). This observation indicated that the formation of the A state may be the initial step in the misfolding pathway. We characterized the structure of the A state using circular dichroism, hydrogen/deuterium exchange coupled with NMR, etc. and found that the Strand 1-Helix 1-Strand 2 segment was completely unfolded in the A state, whereas the Helix 2-Helix 3 segment retained a native-like helical structure. Our studies revealed how the native structure is altered during the early stage of misfolding and how the misfolding pathway is related to the native folding pathway of PrP.

O-05

A local conformation of natively disordered yeast prion monomer determines interspecies prion transmissibility

Toshinobu Shida[1,2], Yuji O. Kamatari[3], Kazuo Kuwata[4], Motomasa Tanaka[1,2]

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Prions are infectious agents that cause fatal neurodegenerative disorders in mammals. While prion transmission between different species is very limited, a molecular mechanism underlying the species barrier remains largely unclear. Here, we used the yeast prion Sup35NM as a model protein from two highly divergent yeast species, S. cerevisiae (SC) and K. lactis (KL), and exploited several biophysical and yeast genetics approaches to investigate how prion monomer dynamics affect the interspecies prion transmission.

First, we used nuclear magnetic resonance (NMR) spectroscopy to assess the molecular motions of SC and KL Sup35NM monomers. A variety of NMR analyses revealed that only a small segment of the protein showed a remarkably different protein motion between the two Sup35NM monomers. To examine whether this difference plays a role in prion transmission between the two species, we prepared a SC-based Sup35NM chimera protein by replacing this short segment with corresponding amino acid residues in KL Sup35NM and performed a cross-seeding assay using an amyloid-specific dye, thioflavin T. Strikingly, the SC chimera monomer interacted with KL Sup35 amyloid as effectively as KL Sup35NM monomer. Furthermore, we found that the replacement of several amino acids was sufficient to confer the SC Sup35NM protein with a high cross-seeding reactivity toward KL Sup35NM amyloids. The cross-seeding was also confirmed in vivo by cytoduction experiments. Finally, we performed NMR analyses in order to examine whether these mutations alter SC Sup35 monomer dynamics. Surprisingly, the mutations in the SC Sup35NM chimera were sufficient to induce the formation of the local conformation that KL Sup35NM adopts.

Taken together, the present study revealed that a local conformation of natively disordered Sup35NM is crucial for breaking the prion transmission barrier.

P-009

Calibration of ultrasonic power and conformational analysis of MoPrP amyloid fibrils

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Ultrasonication has been widely used to amplify the scrapie from of prion protein, or other amyloids *in vitro*. Firstly, to elucidate the effects of ultrasonication on the formation of amyloid fibrils, we determined the ultrasonic power using both calorimetry and KI oxidation. These methods revealed that the ultrasonic power in our system was ranged from 0.3 W to 2.7 W. Based on the proper calibration of the ultrasonic power, the amyloid formation of MoPrP was investigated. The nucleation time of amyloid fibrils was found to be shortened almost proportionally to the ultrasonic power, indicating that the probability of the occurrence of nucleus formation increases proportionally to the ultrasonic power. Although amyloid fibrils were formed early at the strong ultrasonic power larger than 2.6 W, fine fragmentation of amyloid fibrils occurred. Thus, a balance between the extension and the fragmentation of the preformed amyloid fibrils is essential.

Subsequently, to characterize the conformation of MoPrP fibrils formed under proper ultrasonic irradiation, we synthesized position-specific double-fluorescence labeled MoPrP for a FRET analysis. The result indicated that a distance between fluorescence labeled N- and C-terminal sites of MoPrP increased upon the formation of amyloid fibrils compared with that of the native state. These approaches proposed here, calibration of ultrasonic power using calorimetry/KI oxidation methods and conformational analysis using FRET spectroscopy, are useful for amplifying prions *in vitro* and elucidating the conformation of abnormal PrP, respectively.

P-019

Discovery of anti-prion agents using a PyMOL plugin-based logical drug design platform NAGARA

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Logical drug design is a strategy for designing small compounds called medical chaperones (MCs) that stabilize the conformation of a target protein. To accelerate the design procedure, we developed a plugin called "NAGARA" based on the PyMOL program, and applied it to the discovery of MCs that stabilize the cellular form of a prion protein (PrP^C). In NAGARA, we constructed a single platform to unify the docking simulation (DS), free energy calculation by molecular dynamics (MD) simulation, and interfragment interaction energy (IFIE) calculation by quantum chemistry (QC) calculation. NAGARA also enables large-scale parallel computing via a convenient graphical user interface. Here, we demonstrated its performance and its broad applicability from drug discovery to lead optimization with full compatibility with various experimental methods including Western blotting (WB) analysis, surface plasmon resonance (SPR), and nuclear magnetic resonance (NMR) measurements. Combining DS and WB, we discovered anti-prion activities for two compounds and tegobuvir (TGV), a non-nucleoside non-structural protein NS5B polymerase inhibitor showing activity against hepatitis C virus genotype 1. Binding profiles predicted by MD and QC are consistent with those obtained by SPR and NMR. Free energy analyses showed that these compounds stabilize the PrP^C conformation by decreasing the conformational fluctuation of the PrP^C. Because TGV has been already approved as a medicine, its extension to prion diseases is straightforward. Finally, we evaluated the affinities of the fragmented regions of TGV using QC and found a clue for its further optimization. By repeating WB, MD, and QC recursively, we were able to obtain the optimum lead structure.

P-048

Classification of anti-prion compounds based on the binding properties to prion proteins

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To date, a variety of anti-prion compounds have been reported that are effective in ex vivo and also in vivo treatment experiments. However the molecular mechanisms of most of these compounds remain unknown. Here we classified anti-prion mechanisms into four categories; I: conformational stabilization, II: interference with the interaction between the cellular form of prion protein (PrPC) and the scrapie form (PrPSc), III: precipitation of prion proteins, and IV: interaction with proteins other than PrPC (1). To characterize the anti-prion compounds according to this classification, we determined their binding affinities to PrPC and their binding sites in PrPC using the surface plasmon resonance and NMR spectroscopy, respectively. GN8 (2), GJP49 (3), TGV (4) bind specifically to the hot spot in PrPC, and act as 'medicinal chaperones' to stabilize the native conformation and interfere with the interaction between PrPC and PrPSc. Thus mechanism I & II are predominant. While quinacrine and epigallocathechin bind to PrPC rather non-specifically. They may mainly interfere with the intermolecular interaction, and mechanism II may be applied. RNA aptamer R12 binds specifically to the N-terminal region of PrPC and has anti-prion activity (5), which is also categorized into the mechanism II. On the other hand Congo red and pentosan polysulfate bind to PrPC and cause its precipitation reducing the effective concentration of prion protein. So, mechanism III is appropriate. Finally CP60, an edarabone derivative, FK506 (6) etc. never bind to PrPC but may interact with PrPSc or other relevant proteins, and can be explained by mechanism IV, whose details must be elucidated further. The proposed characterization of diverse anti-prion compound would help understanding their anti-prion activities as well as facilitating further effective anti-prion drug discovery.

References: 1. Kamatari et al., Protein Sci. 22, 22-34, 2013. 2. Kuwata et al., Proc. Natl. Acad. Sci. U. S. A. 104, 11921-6, 2007. 3. Hosokawa-Muto et al., Antimicrob. Agents Chemother. 53, 765-71, 2009. 4. Ma et al., Biochem. Biophys. Res. Commun., 469, 930-5, 2016. 5. Mashima et al., Nucleic Acids Res. 37, 6249-58, 2009. 6. Nakagaki et al., Autophagy 9, 1386-94, 2013.

P-059

Latent structural variation in a yeast prion monomer determines strain phenotypes

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A remarkable feature of neurodegenerative diseases is that mutations in causal genes or discrete cellular conditions can trigger the formation of distinct amyloid conformations that can undergo alternate cellular physiological trajectories such as cytotoxicity or tissue specificity. However, how structural polymorphism in amyloid is formed from a monomeric protein is poorly understood. Here, we reveal the atomic basis for structural polymorphism in a monomeric protein and its physiological consequences for amyloid formation and cellular phenotype. Sup35NM, an N terminal fragment of Sup35, the protein determinant of [PSI+] prion yeast, can form two alternate amyloid core conformations. Using biophysical methods, we revealed that solvent-exposed residues are crucial determinants of alternate amyloid core regions. Long-range interactions in the prion domain allowed the formation of local compact structures, which regulate self-intermolecular interactions required for initiation of amyloid formation. The molecular cascade of structural determinism explained the existence of phenotypically distinct [PSI+] strains. Together, these results demonstrate that natively disordered protein monomers contain latent compact local structures that when disinhibited can drive alternate amyloid conformations, redirect chaperone-mediated fiber fragmentation and alter prion strain phenotypes. Therefore, conformational variation in intrinsically disordered monomeric proteins is a critical checkpoint for control of pathological outcomes by propagating amyloids.