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JB Reflections and Perspectives

Nobuhiko Katunuma: an outstanding scientist in the field of proteolysis and warm-hearted 'Kendo Fighter' biochemist

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Professor Nobuhiko Katunuma is well known for his outstanding contribution to the understanding of proteolysis in general and cysteine proteinases and their inhibitors in mammals. In fact, he is a world pioneer in the field. In 1963, he started his highly successful scientific career as a Professor at the Institute for Enzyme Research, the University of Tokushima. During the initial 30 years of his career, he was interested in vitamin B6 metabolism and discovered the acceleration of turnover rates of pyridoxal enzyme in apoprotein formation. After this period, his interest expanded to lysosomal cystein proteinases and their endogenous inhibitors. After determining the crystal structure of human cathepsin B, he generated a series of chemically synthesized specific inhibitors of cathepsins. These inhibitors are currently used throughout the world and some of them have been applied therapeutically in various diseases. During his career and even at present, Professor Katunuma has been studying Biochemistry in Medicine and also practicing to become a 'Kendo sword fencing Fighter'.

Keywords: Cathepsins/protease inhibitors/structural-based new drug design/vitamin B6 metabolism.

Abbreviations: CA-074, *N*-(L-3-trans-propylcarbamoyloxirane-2-carbonyl)-L-isoleucyl-L-proline; CLIK-148, *N*-{L-3-*trans*-[2-(pyridine-2-yl)-ethylcarbamoyl}-Phe dimethylamide; DLFZ, double layer fluorescent zymography; E-64, *N*-[*N*-(L-3-trans-carboxyoxirane-2-carbonyl)-L-Leucyl]agmatine; QPRT, quinolinate phosphoribosyl transferase.

Nobuhiko Katunuma was born in Nagasaki in 1926 and graduated from the School of Medicine at Nagoya University in 1953. After completing his PhD thesis in medical sciences, he went to Sweden to do his postdoctoral training at the Nobel Institute in Stockholm, working with Professor Hugo Theorell. In 1959, he became an Associate Professor at the Institute of Protein Research, Osaka University. In 1963, he moved to the Institute of Enzyme Research, School of Medicine, Tokushima University, where he established his successful scientific career. He became the Director of the Institute since 1971 and also served as Dean of the Medical School between 1980 and 1982. In 1992, he retired from Tokushima University and then moved to the Institute for Health Sciences, at Tokushima Bunri University.

During the initial period of nearly 30 years at Tokushima University, he was interested in the enzymes involved in vitamin B6 metabolism and their intracellular protein turnover. His outstanding work includes the discovery of mitochondrial glutamic-oxalacetic transaminase and the urea cycle glutaminase isoenzymes with his colleague Mitsuko Okada, as well as their roles in hepatocarcinogenesis with his colleague Yasuhiro Kuroda. Regulation of the urea cycle was further investigated by his colleague Takeyori Saheki. In 1971, he discovered the acceleration of pyridoxal enzyme turnover in vitamin B6-deficient animals, and the degradation protease of the apoproteins with his colleague Eiki Kominami. These discoveries shed light on the process of initiation of protein degradation by apoprotein formation in proteolysis. This concept stimulated research on the initiation of various biological events by limited proteolysis, such as histamine release by mast cell chymase, prothrombin activation by mast cell tryptase and initiation of influenza virus entry by tryptase Clara, work that was completed in collaboration with his colleague Hiroshi Kido. In the later period of his work at Tokushima University, he also established a large research project studying the role of lysosomal enzymes and their inhibitors in intracellular proteolysis with his colleagues Takae Towatari and Eiki Kominami. His studies on the crystal structure of human cathepsin B in collaboration with Robert Huber from the Max-Planck-Institute opened the way for his subsequent studied on chemically synthesized specific-inhibitors against cathepsins. In this meaning, he is one of pioneers in the field of structural based drug design.

In 1992, Professor Katunuma retired from Tokushima University and started a second career as Professor and Director of the Institute of Health Sciences, Tokushima Bunri University. From 2000 to 2006, he was appointed as the President of Tokushima Bunri University. In this new position, he participated in the development of new synthetic cysteine proteinase inhibitors, the derivatives of E-64 and the CLIK inhibitors, and studied the role of cysteine proteases in

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bone resorption and antigen presentation. The list of his publications continues to grow even at present fueled by his enthusiasm to science. In this perspective, we summarize his work in the field of proteolysis.

Studies on limited proteolysis as an initiation signal of cell biology

What is the initial signal(s) of intracellular protein degradation? There is no definite answer at present to this key question. Professor Katunuma first suggested that apoprotein formation is one of the key signals for the degradation of vitamin B6 enzymes and identified their limited proteolysis by serine proteases (*1*–*6*). This concept contributed tremendously to the subsequent discoveries of initiation on protein degradation, such as protein phosphorylation and conformational change of proteins by molecular chaperones. Furthermore, the view point of limited proteolysis as a signal in cell biology other than the blood coagulation system prompted many researchers from around the world to investigate various limited proteolysis events. One of the typical events is the regulation of influenza virus entry into cells by tryptase Clara discovered by his colleague Hiroshi Kido (*7*, *8*). Since the influenza virus genome does not have the processing proteases for the viral membrane fusion glycoprotein precursors, entry of this virus into the cells is determined primarily by host cellular, trypsin type, processing proteases that proteolytically activate the fusion glycoprotein precursor of the virus. Other studies revealed the presence of intracellular serine protease inhibitors as regulators of cell signaling through inhibition of serine proteases (*9*–*14*). Figure 1 is a photograph of the top world scientists working in the field of proteolysis who participated in the First International Symposium on Proteinase Inhibitors, which was held in Tokushima in 1982.

Studies on cysteine proteases and their inhibitors

In the field of intracellular protein catabolism, Professor Katunuma was interested in lysosomal cysteine proteinases and their endogenous inhibitors. This interest was initialed by the discovery by his colleague, Takae Towatari, of a new cathepsin in a fraction of cathepsin B1 isolated from rat liver lysosomes in 1976 (*15*). This enzyme became known later as 'cathepsin L' (*16*, *17*). Professor Katunuma and his collogues first succeeded in crystallizing cathepsin B in mammalian cysteine proteases and then determined the amino acid sequence (*18*–*20*). These studies further developed to cDNA cloning by Kazumi Ishidoh (*21*, *22*) and the amino acid sequence determination of other related cathepsins (*23*, *24*). In the studies on the regulation of intralysosomal protein degradation, he and his colleague, Eiki Kominami, discovered endogenous cysteine protease inhibitors namely cystatins (*25*–*27*) and studied the mechanisms that regulate the inhibitory activities. They later reported that the inhibitory activity of cystatin B is regulated by oxidation and

Fig. 1 Participants of the International Symposium on Proteinase Inhibitors, held in 1982 in Tokushima and organized by Professor Katunuma.

reduction (*28*). Cystatin B dimmer forms large aggregates on the membrane, which are closely related to the senile plaque formation in the brain of various degenerative diseases (*29*, *30*). His studies on cysteine proteases and their regulation by cystatins, led to a fruitful collaboration with Vito Turk, from Jozef-Stefan Institute in Slovenia, who was regarded as a 'friendly competitor', and the two established a strong relationship that stimulated their research interests. In collaboration with Vito's group, Professor Katunuma established the crystal structure technology and developed the 'structural-based inhibitor design for cysteine proteinases' (*31*, *32*). Figure 2 is snapshot of a beer party in Germany with his friends taken in 1990.

Development of specific inhibitors of cathepsin B, L, H and K

E-64 was originally isolated from *Aspergillus japonicus* and its analogues inhibit various papain-type cysteine proteases (*33*–*36*). Professor Katunuma succeeded in synthesizing specific inhibitors of cathepsin B, L, H and K, including CA-074 for cathepsin B (*37*, *38*), CLIKs-148 and -195 for cathepsin L, CLIK-060 for cathepsin S, and CLIKs-164 and -166 for cathepsin K (*39*–*41*). The mechanism responsible for the selective inhibition of cathepsin B by CA-030 was investigated by analysing the co-crystal structure of cathepsin B and CA-030 (*42*). Furthermore, the inhibition mechanism of CLIK148 was revealed by the co-crystal structure analysis using papain as a surrogate cathepsin L

(Fig. 3) by his colleague, Hideaki Tsuge (*40*). Administration of these specific inhibitors to mice with deletion of various genes revealed specific biological functions of cathepsins *in vivo*; the role of cathepsin B in antigen processing through Th2-mediated IL-4 production (*43*); the role of cathepsin L in cancer metastasis through degradation of retinoic acid receptor, TGF-β production and glucose uptake into the cell (*44–47*); as well as the role of cathepsin S in the pathogenesis of Sjögren's syndrome and in the regulation of the amount in MHC class II molecule on the cell surface (*48*, *49*). The combination of these specific inhibitors provides selective measurement of cathepsins *in vivo* using synthetic substrates (*50*).

Development of a new method for detection of protease-interacting proteins in crude samples

In his studies on protein catabolism in laboratory animals, Professor Katunuma established a new method for detection of protease-interacting proteins in crude samples, named 'Double layer fluorescent zymography (DLFZ)' (*51*). The use of this method led to a novel finding on the interaction of quinolinate phosphoribosyl transferase (QPRT), a key enzyme in the *de novo* NAD^+ synthesis pathway, with active-caspase-3 (*52*). Depletion of QPRT protein by siRNA increased spontaneous cell death. These results indicated that QPRT seems to act as a chaperone for spontaneously activated caspase-3. The DLFZ technique is regarded as a powerful tool to search for other protease-interacting proteins.

Fig. 2 Beer party with his intimate friends in October 1990. From left, Vito Turk, Marianne Jochum, Nobuhiko Katunuma, Helmut Holzer, Bonnie Sloane and Tamara Lah.

Epilogue

Professor Katunuma's scientific interest is linked to medical and biomedical research. When Katunuma made up his mind to become a biochemist at a young age, his great uncle, Professor Seizo Katsunuma, the President of Nagoya University, told him 'Biochemists working at Medical Schools should contribute in the fight against diseases in man'. Katunuma's contribution to medical science was recognized through the '1990 National Violet Ribbon Decoration for Scientists and Artists' award. His brain always produces a constant stream of new ideas and his exciting presentations stimulate his colleagues. He always closely watches his students as they execute their experiments and has been involved in many of these studies directly himself. These experiments sharpen his scientific sense and accumulate scientific knowledge. For this reason, you find many of his students gather around him. To date, more than 30 researchers who had worked with Professor Katunuma in the past have established their own laboratories and been promoted to Professors or Laboratory Heads, both in Japan and overseas. His philosophy of science and love for science are securely succeeded to young researchers.

Outside the scientific field, Professor Katunuma is a man with many interests, including music, photography and kendo sword fencing. When he was a child, he started to train himself as a 'Kendo sword

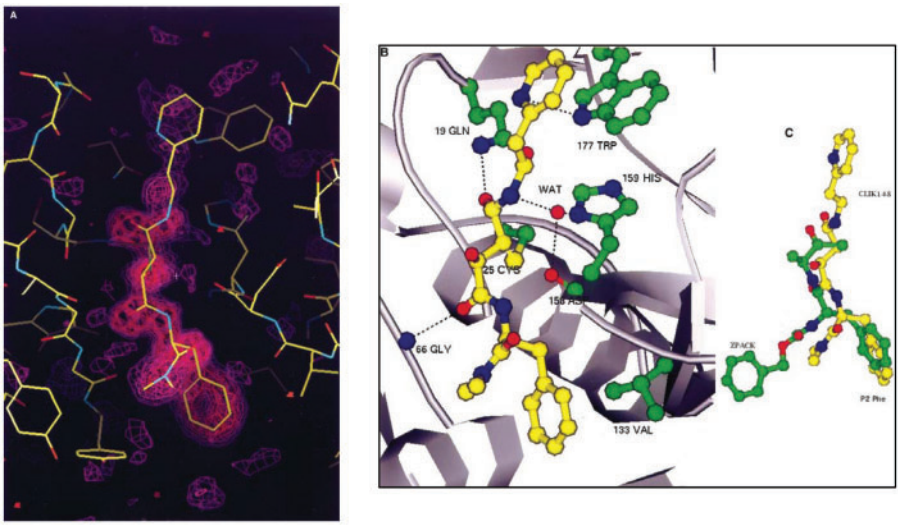

Fig. 3 Co-crystal structure of CLIK-148 and papain as a surrogate cathepsin L. This model is one of the great fruits of the structural-based inhibitor design [reproduced from ref. (*40*) with permission].

fencing fighter' every day. This culminated in receiving the great title of 'Kyoushi' with 'Seventh Master Degree in Kendo'. In July 7 in 2010, Katunuma celebrated his 84th birthday and is still working as a Biochemist in Medicine with a warm heart to humanity.

Conflict of interest
None declared.

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