

Review

Reminiscence of 40-year research on nitrogen metabolism

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(Communicated by Tasuku HONJO, M.J.A.)

Abstract: This article summarizes my research over 40 years. The main theme of my work is nitrogen metabolism of amino acids, though later I focused on protein turnover in the cell. In the first years of my research work, I was busy dissecting the pathways involved in the metabolism of certain amino acids and their related enzymes. Then I became interested in the physiology and regulation of metabolism of these amino acids. For that, I used primary cultured hepatocytes, which contain many liver-specific enzymes. However, this play field was very rough around 1970 and hence I had to smooth them (differentiated) first. We discovered a specific growth factor (hepatocyte growth factor, HGF) in rat platelets. Exceptionally, I also worked on branched chain amino acids (valine, leucine and isoleucine). These amino acids are not efficiently metabolized in the liver, so I had to consider the physiology of extrahepatic tissues as well. Finally, I came across a huge protease complex, the proteasome. Whether these players, small amino acid metabolizing enzymes and the huge protease complex, danced well in harmony on my playground or not, I still do not know.

Keywords: amino acids, metabolism, hepatocytes, HGF (hepatocyte growth factor), proteasome, BCAA (branched chain amino acids)

I graduated from Osaka University Medical School in 1952 and enrolled in the Graduate School to work with Dr. Masami Suda in the Institute of Microbial Diseases. Dr. Suda was an Assistant Professor though there was no vacant Professor position in the laboratory. He was young and active in his research on protein turnover, particularly substrate-induced enzyme induction. Historically, biochemical research in the Medical School was started by Professor Yashiro Kotake in the beginning of the 20th century. He was interested in the metabolism of amino acids, particularly tryptophan and found kynurenin as an intermediate. He also discovered urocanase as the first enzyme to metabolize histidine. His findings were appreciated worldwide at that time and he was asked to contribute to a chapter on amino acid metabolism in volumes 3 and 4 (published in 1934 and 1935, respectively) of the Annual Review of Biochemistry. In those days, research in biochemistry was not very well regarded in Japan and none had been asked before to write a review. Consequent to this interest, research on

nitrogen metabolism flourished in the Medical School. When I started my work, many were conducting research on the so-called β - or γ -elimination by bacterial enzymes. These were release of the last part of amino acid carbons, producing phenol, indole, or methyl mercaptan from tyrosine, tryptophan and methionine, respectively. My first task was to purify the cysteine desulfhydrase of bacteria. At that time, it was thought that cysteine was metabolized to ammonia, hydrogen sulfide and pyruvate by a single-step reaction. However, Dr. Suda suspected the following single-step mechanism:



He anticipated a two-step reaction through an intermediate compound. Working with him, I used to culture soil bacteria every day and prepare cell free extracts, then measure the reaction products mentioned above to construct the kinetic curve. However, the research conditions at that time were tough; *e.g.*, high-quality biochemical reagents, especially isotopes, and cold centrifuges were not available commercially. So you can imagine that when I read the journals at that time, I envied the progress of work in the USA. Even now, when I smell hydrogen sulfide, I recall vividly and sentimentally the poor

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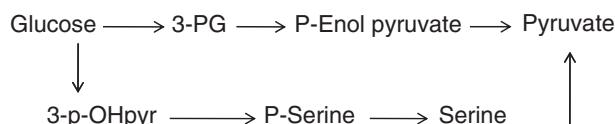
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research conditions. In essence, I did not achieve much in my research work in the first two years, but we always discussed eagerly though sometimes meaninglessly our research and how to improve it. At that time, there was already the concept of “one gene—one enzyme theory”, but there was no molecular evidence of gene. The most common tools used to measure enzyme activity were the Warburg’s manometer and colorimetric photometer. Furthermore, proteins were usually purified with acetone or ammonium sulfate and there was certainly no column fractionation.

Then in 1954, I was lucky to be given the opportunity to work in the USA. My second mentor, Dr. Yoshiro Takeda, who was a research assistant working in Dr. Suda’s laboratory, left Japan to work in the USA two years earlier and while there he helped me find work at the University of California/Berkeley, Medical School, Department of Physiological Chemistry under the supervision of Professor D.M. Greenberg. It was quite unique under such poor research conditions in Japan that Dr. Suda always encouraged us to publish our work in English and to visit US laboratory, although he himself had never been abroad. While all this is not unusual today, but in the fifties of the last century, it was really difficult to write papers in English and to go to the USA. Therefore, I always consider Dr. Suda as a remarkable teacher for young students and I am grateful for his help and vision.

Doing research and living in the USA were wonderful. In the laboratory where I worked, everything was available to do research and the academic environment was marvelous. There were many distinguished scientists and we always had hot scientific discussions. Professor Greenberg was interested in the so-called “active one carbon unit”, which was produced *via* tetrahydrofolic acid-mediated donors, mainly from serine or glycine. He suggested that pyruvate could be converted to serine *via* transamination. He also advised me to use animals only in my research rather than bacteria. This was his policy in the laboratory. Accordingly, I started to use [^{14}C] pyruvate to measure the formation of serine in rat liver homogenate, but naturally the product was always alanine. Therefore, I thought that I should start from glucose as the original source of carbon for serine production. Indeed, [^{14}C] glucose was incorporated into isolated serine. Next, I looked for possible precursor from glycolytic pathway and thought that it might be 3-phosphoglyceric acid (3-PG). Dr. Greenberg suggested that I should visit

Professor Calvin in the Photosynthesis Laboratory. Professor Calvin had many paper-chromatograms that contained various intermediates from [^{14}C] CO_2 to carbohydrates. I felt that this was a real university where I could obtain a lot of information from the people surrounding me. I was able to obtain numerous chromatography papers and extracted [^{14}C] 3-PG. In the next step, I incubated this extract with rat liver homogenate. Then I found [^{14}C] serine as a product. It was midnight in August 1955 and I remember dancing around with tremendous joy in the laboratory. My discovery happened just at the end of my first year in the USA and I was in anxious to get good results. Subsequently, I proposed that this 3-PG could be oxidized to 3-P-hydroxy pyruvate (3-P-OHpyr) followed by transamination to form phosphoserine (P-serine). This could be converted to serine by a phosphatase (see the pathway below).



Delighted and happy, I worked during the second year to determine the dehydrogenase, transaminase and phosphatase activities in the partially purified liver extracts.¹⁾ The methods I used were mostly paper chromatographies and colorimetry. The results clearly showed that serine could be formed from 3-PG, which is an intermediate of glycolysis. At that time, everyone was getting excited about phosphorylation of proteins in biological specimens since none had shown before the presence of free P-serine in living material. It was also unique to find phosphorylated intermediate in amino acid metabolism. My work was accordingly appreciated in the field of metabolism research. Around the same time, Dr. H. J. Sallach identified a non-phosphorylated pathway through D-glycerate and subsequent research showed that in fact both pathways were functional depending on animal species and bacteria.²⁾ Dr. Greenberg published a series of monograph reviews on the “Metabolic Pathways”, and my work was cited in many papers.²⁾

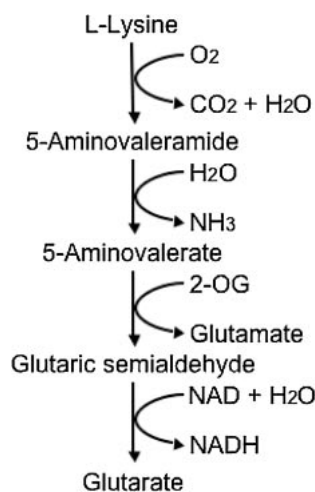
In my third year (1956) in the USA, I moved to the McCollum-Pratt Institute at Johns Hopkins University in Baltimore, because I thought that I should gain new experience. Research in this institute was very active at that time and the Director of the Institute, Dr. W. McElroy, organized annually a high-caliber international symposium, under the title of ‘Phosphorylation’ or ‘Nitrogen Metabolism’. Dr.

McElroy's Institute provided more freedom and some of the people who worked in that Institute later became world authorities in their fields such as Drs. N. Kaplan, S. Colowick, and F. Kenny. With regard to scientific research, Dr. McElroy reported that luciferase activated by ATP produced bioluminescence, like in firefly. He suggested that I work in this field, but I thought immaturely that the medical field does not use bioluminescence and instead I decided to focus on detoxification of hydrogen sulfide to produce thiosulfate ($\text{H}_2\text{S}_2\text{O}_3$) in animals. This decision was based on my nostalgic idea on sulfur metabolism, which I used to work with. In animal, thiosulfate is excreted following inhalation of hydrogen sulfide. However, this work did not progress much within one year and I found many non-enzymatic reactions around hydrogen sulfide.

In September 1957, I returned to Osaka and was appointed a Research Assistant at the Department of Nutrition, School of Medicine, with Dr. Suda, who had been at that time promoted to a Professor. Again, I was lucky for being able to secure a position as soon as I returned to Japan. I say this because I know of the suffering of young graduates nowadays in finding new posts. After coming back to Japan, the Japanese Society of Biochemistry organized the International Symposium of Enzyme Chemistry in Tokyo and Kyoto, which represented the first international meeting of biochemistry in Japan. The symposium was attended by several famous scientists from around the world, who delivered excellent lectures followed by heated discussion and resulted in the publication of excellent papers.³⁾ The symposium program also included several satellite meetings including amino acid metabolism (Photo. 1). Interestingly, the symposium provided the chance for young Japanese biochemists to obtain work abroad.

Dr. Suda suggested that I start my work on bacterial metabolism involving lysine oxidation, including the production of 5-amino valeric acid, ammonia and CO_2 in a single-step reaction, which was also anticipated to include an oxygenase enzyme. Accordingly, I purified the enzyme and collaborated with Professor Osamu Hayaishi, who was appointed as Professor of Biochemistry at Kyoto University Medical School at that time, and we showed that this enzyme was probably an oxygenase whose function was to oxidize lysine. Professor Hayaishi was an expert in oxygenation and employed [^{18}O] gas in his experiments. Indeed, our work showed the incorporation of this molecular oxygen to produce 5-aminovaleric acid. Later, his group showed that such lysine

oxygenation was a two-step reaction and involved the production of 5-aminovaleramide.^{4),5)} (see the reaction pathway below)



In 1959, I was appointed Assistant Professor of Biochemistry, Dental School of Osaka University. This School had been established newly and chaired by Professor Yoshiro Takeda, who was my second mentor and since then we were very close friends. Later, we both moved to Tokushima independently, where I was appointed a Professor at the Medical School while he became the first Dean of the Dental School. Both of us worked in the medical field in the past and had no experience in the dental field. There was very little biochemical work in the dental field at that time in Japan. Professor Takeda found a report describing the isolation of liver cells by mechanical dispersion of perfused liver. Accordingly, we used this method to study metabolic regulation in the rat liver. At that time, we became more interested in metabolic regulation such as hormonal control of gene expression than enzyme characterization, although molecular biology was still not popular at that time. Microscopic examination of the isolated cells showed the beautiful round cells, but we later found that these isolated cells were actually cells damaged by mechanical dispersion and almost all soluble enzymes leaked out from the cells during this process, and therefore we were not able to show any metabolic activity in that preparation. Of course, that included no response to any of the hormones tested in these damaged cells. This waste of time and effort was entirely due to our ignorance of the intricate field of cell culture and cell biology. We struggled for several years to revive these dead cells to no avail. Based on my experience and research failure in the isolated liver cells, I learnt a lot about

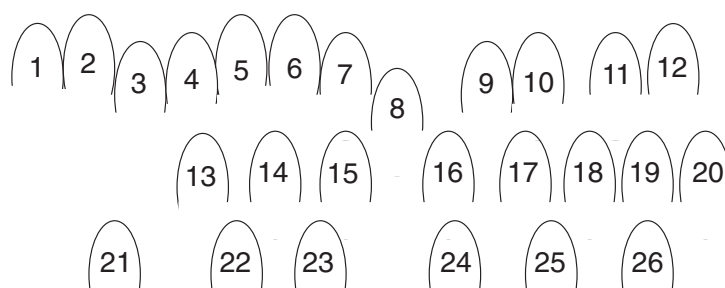


Photo. 1. Photograph of participants at the International meeting on amino acid metabolism held in Osaka, 1957. 1. Y. Takeda (Osaka), 2. Y. Sakamoto (Osaka), 3. M. Suda (Osaka), 4-6. Taiwan participants, 7. H. Wada (Osaka), 8. S. Yoshimatsu (Nara), 9. A. Ichihara (Osaka), 10. Y. Masai (Osaka), 11. O. Hayaishi (Bethesda), 12. J. Matsumoto (Osaka), 13. Z. Matsuoka (Osaka), 14. Y. Sera (Osaka), 15. Y. Kotake (Wakayama), 16. O. Wiss (Basel), 17. B. Magasanik (Boston), 18. W.E. Knox (Boston), 19. E. E. Snell (Berkeley), 20. A. E. Braunstein (Moscow), 21. R. Hirohata (Ube), 22. S. Ueda (Osaka), 23. Y. Kotake (Wakayama), 24. K. Ichihara (Osaka), 25. S. Otani, 26. C. Fromageot (Paris).

cell biology. Although established cultured cells are useful for specific work, they resemble cancer cells in that they do not show normal metabolic activity and also we wanted more differentiated cultured cells.

In 1965, I was appointed a Professor of Enzyme Pathology at the Institute of Enzyme Research, Tokushima University Medical School. Among the several projects available to study, I chose the characterization of transamination of branched chain amino acids (BCAA, *i.e.*, valine, leucine and isoleucine). Whether the transaminase was specific for the three amino acids or each amino acid had a specific transaminase was unknown at that time. I found that the enzyme was fairly specific for BCAA, although methionine was a substrate with low activity.⁶⁾ It was interesting that around the same time, Dr. W.T. Jenkins in the USA reported similar findings. His enzyme was more highly purified than mine, but I showed a unique tissue distribution of this enzyme in rats.⁷⁾ Usually the liver shows high levels

of amino acid metabolism due to its gluconeogenic nature, however BCAA transaminase is extremely low in the liver, compared with moderate activity in the muscle and heart, although their products, keto acids, are degraded in the liver. This unique tissue distribution is interesting, and later I found the highest activity in the pancreas and stomach⁸⁾ (Table 1). Although the reason for the high enzyme activity in the stomach and pancreas remains as a mystery, it could be a signal for digestive juice secretion. I later identified two types of the enzyme (isozymes), including their characterization immunologically⁹⁾ (see Table 1). I also found that the isozyme pattern in the rat hepatoma cells was different from that in the normal liver. BCAA are unique physiologically compared with other amino acids. Although they are essential in animal nutrition, they are not metabolized in the liver, but do so mostly in the extrahepatic tissues, perhaps in the muscle. Muscles constitute about 50% of body weight, hence although

Table 1. Tissue distribution of BCAA transaminase isozymes

	Activity (unit)	Rat	Human
		Isozyme I (%)*	
Liver	10	100	80
Lung	18	100	
Kidney	139	100	90
Skeletal muscle	50	100	90
Brain	74	30	20
Heart	148	100	90
Stomach	820	100	90
Pancreas	1250	100	60
Ovary	50	70	50
Lactating mammary gland	100	100	

*Rest of the percentage is type III.

BCAA isozymes are classified into types I, II, and III. Type I and III are mainly localized in mitochondrial and cytosolic fractions, respectively. Type II is currently assumed to be identical to asparagine aminotransferase. In general, isozyme III is increased in various transformed cells.

the BCAA transaminase activity is not very high in the muscle, the total metabolizing activity must be the highest in body. BCAA also constitute a high percentage of the body proteins in general. Leucine is used at high proportions for energy production among other amino acids as well. It is also a potent stimulator of insulin secretion. Therefore, BCAA may be used as an energy source in animals. Based on these considerations, it is interesting that BCAA, particularly leucine, are effective in activation of protein synthesis and inhibition of protein degradation both *in vivo* and *in vitro*.¹⁰⁾ However, the mechanisms responsible for the effects of BCAA on protein turnover remain poorly understood. Interestingly Dr. K. Yonezawa's group used cultured cells and reported that leucine stimulates mammalian target of rapamycin (mTOR) kinase, which is an upstream regulator of the ribosomal S6 protein kinase 1 and 4E-BP1 (eIF4E binding protein 1), and hence mRNA translation.¹¹⁾ Several groups showed that BCAA prevent liver cirrhosis by providing energy and possible signals to stimulate protein synthesis including production of albumin and hepatocyte growth factor (HGF).¹²⁾ Clinically, Dr. T.E. Fischer proposed the importance of blood BCAA/tyrosine and BCAA/phenylalanine ratios, particularly in patients with acute liver encephalopathy,¹³⁾ though I do not know the exact effects and mechanism of action. I contributed to writing a chapter on BCAA in the Reviews of Enzymology and Physiology of BCAA.^{9),14)} In 2004, I organized an International Symposium of the Pathophysiology of BCAA in Tokyo.¹⁵⁾

On several occasions, our findings were similar to those published by other scientists at almost the same time independently. My report on serine biosynthesis was published at the same time when Dr. Sallach published his paper, as explained above. What an incredible coincidence since we had no contact previously. It makes me wonder how many people have the same scientific interest in the same field at the same time who publish their findings at the same time. The same also happened with regard to the BCAA transaminase between my work and that of Jenkins and his group.

In 1969, Berry and Friend reported the use of collagenase to isolate rat hepatocytes that retain their active liver-specific enzymes.¹⁶⁾ I was really keen to use this method in my studies designed to understand liver metabolism. I have worked mostly with liver specific enzymes, but it was the study of the enzymes themselves and the understanding of their physiology that interested me most rather than the regulation of enzyme activity. I considered the method very useful to study hormonal regulation of gene expression at the living cell level. I cannot forget the joy I felt when I found that the addition of glucocorticoid and glucagon to the culture media of hepatocytes isolated using the collagenase method truly induced serine dehydratase and tryptophan dioxygenase, which are liver-specific enzymes, whereas no such induction was noted in mechanically dispersed cells or established liver cells.^{17),18)} We published many papers showing that these primary cultured hepatocytes are really mature liver cells and retain various differentiated liver specific functions such as glyco-/gluconeogenesis, lipogenesis and the respective hormonal responses.¹⁸⁾ Among many markers of liver-specific enzymes, we focused our attention on serine dehydratase gene, because it is a central enzyme for the conversion of amino acid carbons to glucose. This gene was cloned and its molecular sequence was determined.¹⁹⁾ Furthermore, the genetic mechanisms of the active responses of glucocorticoids and glucagon were determined.²⁰⁾ These genetic techniques quickly became popular at that time. In the early period of my work, I was able to measure enzyme activity only while hormonal regulations were studied in hormone-treated animals and we called them gene expressions. However, around the 1970s, we were able to use the cell culture system for hormonal regulation and gene manipulation studies.

Our main interest in hepatocyte research, however, was whether these cells could proliferate *in vitro* in the presence of growth factor. It has been known for

long time that the partially hepatectomized rat liver could proliferate actively and recover the original volume. We showed that there was indeed DNA synthesis of primary cultured hepatocytes in the presence of epidermal growth factor (EGF). Then we searched for a growth factor specific for hepatocytes. We checked for such factors in various rat tissues and found that circulating platelets carry such factor. We isolated platelets from over 5,000 rats and purified the factor. Further analysis showed it was more than 80,000 Da in size and consisted of a heterodimer. We named it HGF. Around the same time, Dr. Toshikazu Nakamura who was one of the most active researchers in my laboratory was appointed as a Professor in Science at Kyushu University. Later, he identified the unique structure of HGF.²¹⁾ He also found that HGF was not only a liver growth factor, but also played important roles in diverse cellular functions such as angiogenesis, morphogenesis or anti-liver cirrhosis activity. Dr. Nakamura then moved to Osaka University and is still currently in the same University, where he reported that one domain of HGF (NK4), which functions as a specific inhibitor of HGF, is a potent anti-tumor drug.²²⁾ After he left Tokushima, I became interested in the relation between hepatocyte function and morphology of the cells in primary cultures. It is common to use monolayer cultures to examine the cell activities, but this is quite an artificial setup and it should be more natural to mimic the *in vivo* state. Several studies have described more natural culture conditions such as coating dishes with extracellular substratum, addition of various hormones or nutrients.²³⁾ I found an interesting report using positively charged culture dishes, in which cells aggregate to become round and detach from dishes to float in the medium.²⁴⁾ The cultured spheroid-shaped rat hepatocytes have similar morphological features (Fig. 1). This technique yielded some remarkably interesting results. Glucagon, insulin and glucocorticoids regulate the expression of liver-specific enzymes in spheroid cultures more natural than in monolayer cultures. For example, glucokinase is upregulated while hexokinase is downregulated in spheroid cultures²⁵⁾ (Fig. 2), as if hepatocytes are mature cells at resting condition. Interestingly, the transfer of the spheroid cultured cells to monolayer cultures resulted in the gradual reversal of the expression; *i.e.*, downregulation of glucokinase and upregulation of hexokinase. The latter pattern is similar to that seen in hepatoma cells. Of course, the cultured hepatocytes were not cancerous cells in both the monolayer and spheroid cultures, but they seem to transform into the G1 state of the cell

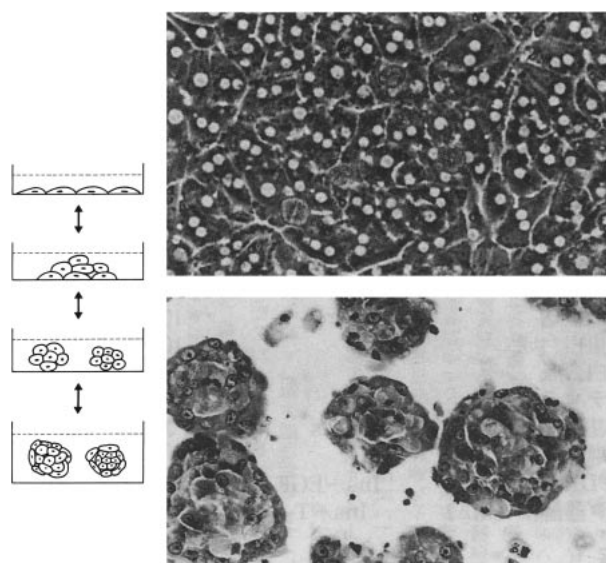


Fig. 1. Changes in the morphology of cultured rat hepatocytes in monolayers (top) and spheroid cultures (bottom).

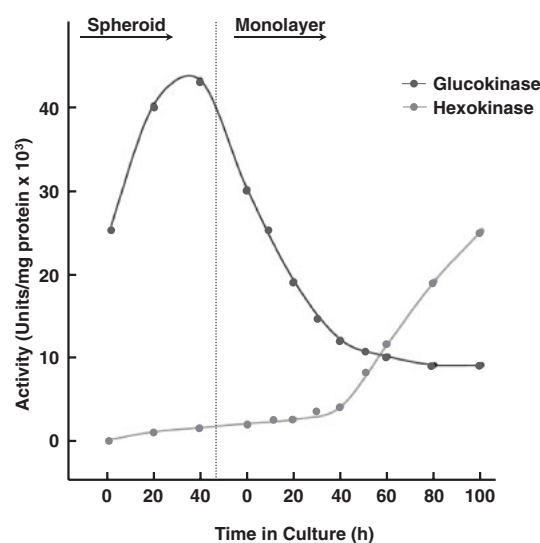


Fig. 2. Activities of glucokinase and hexokinase in spheroid and monolayer cultures of rat hepatocytes.

cycle from a resting state. In spheroid cultures, cells do not proliferate even in the presence of HGF. It is possible that the intracellular structure of the cells in spheroid cultures would generate more natural signals to regulate gene expressions, although this could not have been studied since gene structure analysis was not available. Thus, we considered that the intracellular environment seems more important for signal transduction to gene activation. However, my knowledge of

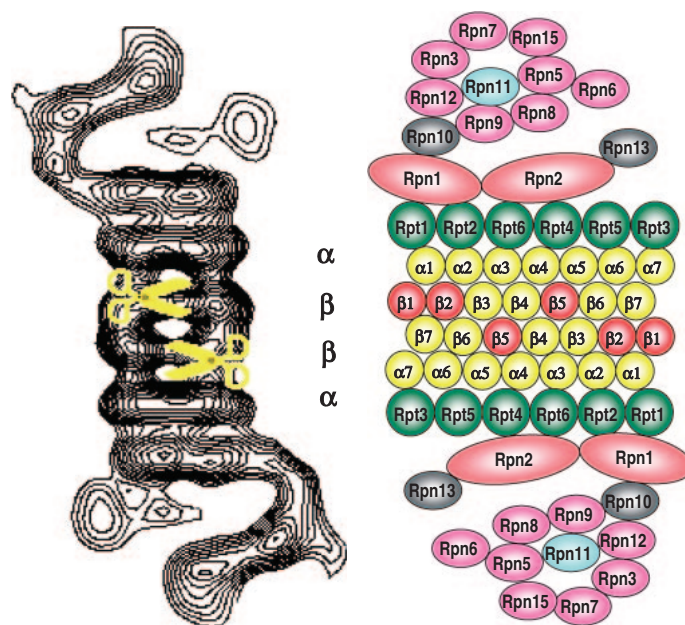


Fig. 3. Schematic diagram of the 26S proteasome complex. *Left panel:* Gross structure (averaged image) of the 20S proteasome based on electron micrography. Photograph kindly provided by W. Baumeister. The α and β rings of the 20S proteasome are indicated. Yellow scissors image the location of two sets of active sites, which are present in the cavity of two β -rings. *Right panel:* Schematic drawing of the subunit structure. The 20S proteasome consists of two outer α rings and two inner β rings, which are made up of seven structurally similar α and β subunits, respectively; the rings form an $\alpha_{1-7}\beta_{1-7}\beta_{1-7}\alpha_{1-7}$ structure. The β_1 , β_2 , and β_5 subunits are associated with the caspase-like, trypsin-like, and chymotrypsin-like activities, respectively. The 19S regulatory particle (RP) caps on both ends of the central 20S proteasomal core. The 19S RP comprises many different subunits that can be subclassified into two groups: Regulatory particle of triple-ATPase (Rpt) subunits and Regulatory particle of non-ATPase (Rpn) subunits.

intracellular structure such as integrin and cytoskeleton was limited to allow me investigate this area. This challenge was also close to my retirement from the University.

At that time, Dr. Keiji Tanaka started working in my laboratory as a research assistant and was interested in protein turnover, because he graduated from the Department of Nutrition at Tokushima University. He was particularly interested in energy required for intracellular protein degradation. From my side, I was curious about the possible involvement of BCAA in protein turnover as mentioned above. Accordingly, we started to look for possible proteases involved in protein degradation. The prevailing concept at that time was that protein degradation served mainly to dispose old proteins and that this process involved proteases such as trypsin and chymotrypsin, though there was little interest in intracellular proteases. Scientists also doubted that protein degradation is an active process that requires energy. Quite the opposite, it turned out to be a very active process involving a large and complex protease. Dr. Tanaka was successful in separating the protease and named it the proteasome. It contained

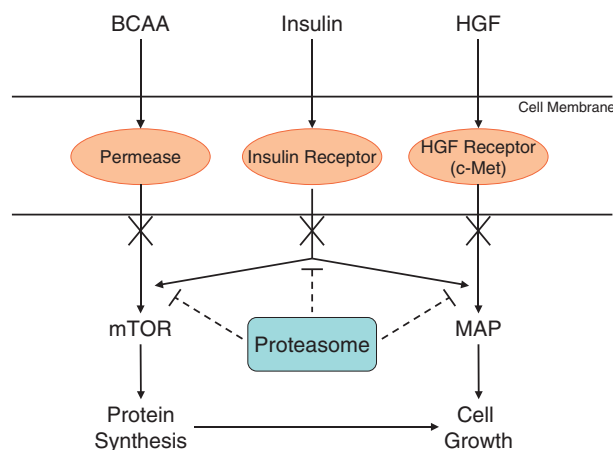


Fig. 4. Proposed relation between BCAA, HGF and proteasomes. For details, see text.

28 subunits with a relative molecular mass of about 750,000. Then, he identified the structure of each subunit by cDNA cloning and showed that these subunits are not-identical (though significantly similar) in amino acid sequence and that they form 4 discs (*i.e.*, $\alpha\beta\alpha$ ring) with 7 subunits each as shown

in Fig. 3.²⁶⁾ This core part (known as 20S proteasome) is attached to the regulatory part on each side to make the larger complex (designated 26S proteasome). In 1996, Dr. Tanaka moved to the Frontier Science Laboratory at the Tokyo Metropolitan Institute of Medical Science. His work had established the role of proteasomes and they were known at that stage to be the main regulators of a multitude of cell activities. Further work showed that the proteasome degrades short-live proteins in the cells, such as receptors of growth factors, factors for cell cycle, transcription factors and MHC class I antigens.²⁷⁾ I should mention here that I did not cite much work of Drs. Nakamura and Tanaka on HGF and proteasomes, respectively, because most of that work was theirs.^{21),22),27)}

I retired from Tokushima University in 1994 and that was the end of my research work. I hope that I summarized my 40 years of work adequately. I know it is not simple or easy, because I did not have a deep philosophy in my work. The first part of my research work followed the advice of my mentor; *i.e.*, characterization of metabolic pathways of amino acids, such as cysteine, serine and lysine. The work on BCAA was purely my own. In general, amino acids are usually used for gluconeogenesis and hence I usually used the rat liver. BCAA degradation is unique compared with the degradation of other amino acids, because they are degraded in extrahepatic tissues. They may become a signal *via* mTOR for protein turnover as suggested in Fig. 4.²⁸⁾ HGF also has a potent insulin action and HGF receptor (cMet) is degraded (regulated) by proteasomes. Proteasomes also regulate BCAA signals. Each of these research projects was conducted separately and independently, but I can firmly say that the entire work conducted over 40 years can be viewed as pieces of a jigsaw puzzle put together to form one big picture. Looking back now I can say that I had a happy and enjoyable 40-year research life. Most importantly, I was surrounded by many distinguished and kind senior and junior researchers and they were the most helpful in my life. I want to thank each one of them for the collaborative and enjoyable work we did together.

Finally, I want to mention the contribution of my dear wife Elizabeth to life science in Japan. She has contributed enormously by editing a number of Japanese papers (including mine) published in English. Elizabeth was born in Cambridge, graduated from Newnham College and obtained her PhD in marine biology. She joined Professor Greenberg's laboratory in Berkeley and then moved to Osaka

with me in 1957. It was just the time during which Japanese life science research had started to match that in other countries and many excellent papers were being published. As most of you know and suffer, all Japanese scientists found it difficult at that time to write papers in English, in addition to the lack of good quality science translators. Elizabeth was the most proper person for that task. It was natural that she found a lot of interest in her editing skills by the Japanese life scientists, and she did help them edit their papers to as many as 400 per year. She realized her importance in this work and valued it much more than her research work. Based on this realization, she gave up work in research and devoted her time to editing. It is my estimate that she edited as many as 10,000 manuscripts written by Japanese scientists during her career. In 1982, Professor Tamio Yamakawa suggested the publication of a book entitled "How to write science papers in English". The book was published by Kyoritsu Shuppan and has become one of the best sellers published by that company; over 31,000 copies have been sold and it is still being reprinted. Elizabeth's motto in science writing was "simple, accurate and clear", and she hated words like "may be" or "suggest". Sadly, Elizabeth died in 2009. Her brother, David and I wrote an obituary in Newnham College Roll Letter in 2009 (800th anniversary of the Cambridge University, p. 214). Also, Dr. Yasuhiro Anraku kindly wrote an obituary in Seikagaku (2009;81:823–825).

Acknowledgements

I thank K. Tanaka and C. Noda for their critical reading and comments on the manuscript.

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(Received Jan. 13, 2010; accepted May 3, 2010)

Profile

Akira Ichihara was born in 1928 at Osaka and graduated the Medical School of Osaka University in 1952 and then entered Graduate School of the Institute of Microbial Diseases. His first work was to characterize cysteine desulhydratase of bacteria and rat liver. From 1954 he spent three years in USA as a postdoctoral fellow, and studied serine biosynthesis in rat liver. In 1957 he came back to Osaka University and characterized transaminase of branched chain amino acids in animal tissues. In 1965 he was appointed as Professor of Enzyme Pathology, Tokushima University. He was interested in differentiated cultured liver cells using primary cultures of rat hepatocytes. Markers of differentiation were amino acid metabolizing enzymes and their genes expression by addition of various hormones. He characterized growth factor of these hepatocytes and named as Hepatocyte Growth Factor (HGF). Final part of his research life till his retirement was to characterize an energy-dependent protein degrading enzyme termed as the proteasome. He retired academic life from a chairman of Enzyme Research Center of the Tokushima University (1994). Therefore, his research as a whole was for characterization of nitrogen metabolism in animals. He served as Presidents of Jpn. Soc. Biochem. (1990) and Jpn. Soc. Cell Biol. (1992). He was an honorary member of Jpn. Soc. Biochem. and Am. Soc. Mol. Biol. Biochem. He was awarded Academic prizes for the Princess Takamatsu Cancer Research and the Uehara Foundation.

