Japan Academy Prize to:

Hiromichi NAGASAWA
Emeritus Professor, The University of Tokyo

for "Bio-organic Chemistry Studies on the Regulation Mechanism of Biomineralization"

Outline of the work:

Minerals such as bones and shells produced by living organisms are called biominerals, and their formation is called biomineralization; calcification is a main type of biomineralization. Biominerals are characterized by the involvement of trace amounts of organic matter.

Carbon dioxide, the principal cause of global warming, accounted for 97% of the atmosphere when the earth was formed; now, it has decreased to only 0.05%. This decrease resulted from the activities of living organisms for 4 billion years since the birth of life and is primarily related to two types of carbon dioxide fixation mechanisms: photosynthesis by green plants and calcium carbonate production by calcifying organisms. Although the detailed molecular mechanism of photosynthesis has been elucidated, the mechanism of calcium carbonate formation remains unclear because the high proportion of inorganic materials made their analysis difficult in life sciences.

Dr. Hiromichi Nagasawa was the first to identify numerous new organic matrices from the calcified tissues of various organisms. By elucidating the role of these organic matrices in calcification, he identified a common mechanism of calcification reactions that transcends species. He contributed significantly to the elucidation of the whole picture.

The presence of organic substances in trace amounts in calcified tissues has long been considered important for calcification reactions; however, they have only been obtained as mixtures, and no purification and analysis of single compounds are performed. To overcome this situation, Dr. Nagasawa successfully purified the main organic matrix from gastroliths of crayfish and cloned a cDNA from its partial amino acid sequences to generate a protein consisting of all 462 amino acid residues (GAMP). This protein has a calcium carbonate binding activity and plays a key role in the formation of gastroliths as a major organic component. Furthermore, he identified major components as organic matrices from calcified tissues of various organisms. However, no common conservation rule was observed in their chemical structures, suggesting that organisms acquired calcification abilities independently during the evolution. In addition to calcification, Dr. Nagasawa has deepened his research on mineral crystallization and has analyzed the control mechanism of biomineralization with a broader perspective.

Studies at the above stage have focused on the organic matrix, which is dominant in calcified tissues, and might overlook the organic matrix that is truly important for calcification. Therefore, Dr. Nagasawa developed completely original assays to detect the trace amounts of biologically active substances related to calcification in vitro, which included calcium carbonate (calcium phosphate) crystal binding activity, crystal polymorph-selective binding activity, chitin binding activity, and amorphous calcium carbonate retention activity. Using these assays, he successfully identified various active trace substances involved in the regulation of calcification.

The spatial arrangements of calcium and carbonate ions in the calcified tissues include calcite (stable at normal temperature and pressure), aragonite and vaterite (both metastable), and amorphous structures. The calcified tissues of various organisms have unique crystal polymorphism. As reported in 1960 that the aragonite polymorphism of pearl oyster nacre is caused by its organic matrix, researchers attempted to identify it, but were unsuccessful. Dr. Nagasawa discovered a trace amount of protein (Pif) that selectively binds to aragonite from crude extracts of nacreous organic substrates by measuring crystal polymorphism-selective binding activity and determined its structure. Further, Pif was proved to induce the aragonite structure *in vitro*. On the other hand, phosphoenolpyruvate and 3-phosphoglycerate were identified as active substances that maintain the amorphous state of calcium carbonate. Following the discovery of quantitatively dominant organic components in calcified tissues, the identification of these organic matrices, which functionally lead to the formation of calcium carbonate with specific configurations, has first elucidated the mechanism of calcification reactions at the material level.

Based on the above results, Dr. Nagasawa proposed an inter-organism common model in which the organic matrices of calcified tissues are classified into insoluble, multifunctional, crystal growth-controlling, and amorphous state-stabilizing matrices. First, an insoluble organic matrix forms a scaffold for calcification, and the specific binding of multifunctional proteins to the insoluble matrix promotes calcium carbonate crystallization and crystal polymorphism. Furthermore, some soluble substrates regulate crystal growth although there is no direct interaction with insoluble organic substrates. A universal model of calcification in organisms based on such experiments is an epoch-making model never proposed before.

Briefly, Dr. Nagasawa has created a trend in the research on calcification mechanism based on the structure and function of organic matrices for calcification reaction, which has not been elucidated so far, thereby making an outstanding contribution to the understanding of the reaction. During this time, he received Young Scientists Award from Japan Society for Bioscience, Biotechnology and Agrochemistry; Japan Prize for Agricultural Science; Yomiuri Prize for Agricultural Science; and the Medal with Purple Ribbon. His record of leading research in this field, including presiding over it, has been highly acclaimed worldwide.

List of Main Publications

1. Ping, L., Nagasawa, H., Matsumoto, K., Suzuki, A. and Fuwa, K. (1986) Extraction and purification of a new compound containing selenium and mercury accumulated in dolphin liver. *Biol. Trace Elem. Res.* 11, 185–199.
2. Ishii, K.,Yanagisawa, T. and Nagasawa, H. (1996) Characterization of a matrix protein in the gastroliths of the crayfish, *Procambarus clarkii*. *Biosci. Biotechnol. Biochem.* 60, 1479–1482.
3. Li, H., Nagasawa, H. and Matsumoto K., (1996) Graphite-Furnace atomic absorption spectrometry of organomercury and organoselenium in extracts of biological samples with an organopalladium matrix modifier. *Anal. Sci.* 12, 215–218.
4. Ishii, K., Tsutsui, N., Watanabe, T., Yanagisawa, T. and Nagasawa, H. (1998) Solubilization and chemical characterization of an insoluble matrix protein in the gastroliths of a crayfish, *Procambarus clarkii*. *Biosci. Biotechnol. Biochem.* 62, 291–296.
5. Tsutsui, N., Ishii, K., Takagi, Y., Watanabe, T. and Nagasawa, H. (1999) Cloning and expression of a cDNA encoding an insoluble matrix protein in the gastroliths of a crayfish, *Procambarus clarkii*. *Zool. Sci.* 16, 619–628.
6. Takagi, Y., Ishii, K., Ozaki, N. and Nagasawa, H. (2000) Immunolocalization of gastrolith matrix protein (GAMP) in the gastroliths and exoskeleton of crayfish, *Procambarus clarkii*. *Zool. Sci.* 17, 179–184.
7. Murayama, E., Okuno, A., Ohira, T., Takagi, Y. and Nagasawa, H. (2000) Molecular cloning and

expression of an otolith matrix protein cDNA from the rainbow trout, *Oncorhynchus mykiss*. *Comp. Biochem. Physiol. Part B* 126, 511–520.

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9. Inoue, H., Ozaki, N. and Nagasawa, H. (2001) Purification and structural determination of a phosphorylated peptide with anti-calcification and chitin-binding activities in the exoskeleton of the crayfish, *Procambarus clarkii*. *Biosci. Biotechnol. Biochem.* 65, 1840–1848.
10. Murayama, E., Takagi, Y., Ohira, T., Davis, J. G., Greene, M. I. and Nagasawa, H. (2002) Fish otolith contains a unique structural protein, otolin-1. *Eur. J. Biochem.* 269, 688–696.
11. Fukuda, I., Ooki, S., Fujita, T., Murayama, E., Nagasawa, H., Isa, Y. and Watanabe, T. (2003) Molecular cloning of a soluble protein in the coral exoskeleton. *Biochem. Biophys. Res. Commun.* 304, 11–17.
12. Inoue, H., Ohira, T., Ozaki, N. and Nagasawa, H. (2003) Cloning and expression of a cDNA encoding a matrix peptide associated with calcification in the exoskeleton of the crayfish. *Comp. Biochem. Physiol.* 136, 755–765.
13. Murayama, E., Takagi, Y. and Nagasawa, H. (2004) Immunohistochemical localization of two otolith matrix proteins in the otolith and inner ear of the rainbow trout, *Oncorhynchus mykiss*: comparative study on the adult inner ear and embryonic otocyst. *Histochem. Cell Biol.* 121, 155–166.
14. Suzuki, M., Murayama, E., Inoue, H., Tohse, H. and Nagasawa, H. (2004) Characterization of a novel matrix protein from the prismatic layer of the Japanese oyster, *Pinctada fucata*. *Biochem. J.* 382, 205–213.
15. Murayama, E., Herbomel, P., Kawakami, A., Takeda, H. and Nagasawa, H. (2005) Otolith matrix proteins, OMP-1 and Otolin-1, are necessary for normal otoliths growth and their correct anchoring onto the sensory maculae. *Mech. Develop.* 122, 791–803.
16. Inoue, H., Ohira, T. and Nagasawa, H. (2007) Significance of the C-terminal acidic region of CAP-1, a cuticle calcification-associated peptide from the crayfish, for calcification. *Peptides* 28, 566–573.
17. Ozaki, N., Sakuda, S. and Nagasawa, H. (2007) A novel highly acidic poltsaccharide with inhibitory activity on calcification from the calcified scale “coccolith” of a coccolithophorid alga, *Pleurochrysis carterae*. *Biochem. Biophys. Res. Commun.* 357, 1172–1176.
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19. Inoue, H., Yuasa-Hashimoto, N., Suzuki, M. and Nagasawa, H. (2008) Structure determination and functional analysis of a soluble matrix protein associated with calcification of exoskeleton of the crayfish, *Procambarus clarkii*. *Biosci. Biotechnol. Biochem.* 72, 2697–2707.
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23. Miyabe, K., Tokunaga, D., Endo, H., Inoue, H., Suzuki, M., Tsutsui, N., Yokoo, N., Kogure, T. and Nagasawa, H. (2012) GSP-37, a novel goldfish scale matrix protein: identification, localization and functional analysis. *Faraday Discuss.* 153, 463–481.

24. Suzuki, M., Iwashima, A., Kimura, M., Kogure, T. and Nagasawa, H. (2013) The molecular evolution of the Pif family proteins in various species of mollusks. *Mar. Biotechnol.* 15, 145–158.
25. Nagasawa, H. (2013) Molecular mechanisms of calcification in aquatic organisms. *Biosci. Biotechnol. Biochem.* 77, 1991–1996.
26. Endo, H., Yoshida, M., Uji, T., Saga, N., Inoue, K. and Nagasawa, H. (2016) Stable nuclear transformation system for the coccolithophorid alga *Pleurochrysis carterae*. *Sci. Rep.* 6, 22252.