In Memoriam: Osamu Hayaishi (1920–2015)

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Dr. Osamu Hayaishi, a world-renowned biochemist and oxygenase scientist, passed away on December 17, 2015, at the age of 95. He was the teacher of the authors of this tribute.

In 1932, a short paper reported “the presence of an oxydase of lipid or lipoxydase of soy seed” (1). Later the “lipoxydase” was crystallized and its properties were described by Theorell et al. (2). Although lipoxydase was the first example of “oxygenase”, its characterization as an oxygenase awaited the epoch-making work in 1955 by the groups of Mason and Hayaishi as will be described below.

Osamu Hayaishi was born in Stockton, CA, in 1920 when his father studied and practiced medicine in the United States. In 1923, his family returned to Japan after visiting Germany for some time. He graduated from Osaka Imperial University School of Medicine in 1942, and then served in the navy as a medical officer until the end of the World War II. When he decided to become a basic researcher, the laboratory conditions were miserable. There were no chemicals or experimental animals, and the research facilities were almost useless. In several works (3–7) written by Hayaishi himself, he vividly and concretely described his life in Japan immediately after World War II.

A famous biochemist at Osaka University, Yashiro Kotake, extensively studied tryptophan metabolism in mammals. His group’s approach was to feed animals with tryptophan and to extract and analyze the urinary metabolites. Their famous contribution was the discovery of kynurenine as a central metabolite, which was further transformed to kynurenic, xanthurenic, and anthranilic acids. Fortunately, Kotake donated several grams of tryptophan to Hayaishi, and this “tryst with tryptophan” (4) was the starting point of Hayaishi’s lifelong research and led him to the discovery of oxygenase.

Pyrocatechase and discovery of oxygenase. Inspired by a paper by Mirick (8), Hayaishi attempted to isolate a soil bacterium that grew on nitrogen with tryptophan as its sole source of carbon, and he obtained a bacterial strain identified later as a pseudomonad. In contrast to mammals, this microorganism metabolized anthranilic acid further to cis, cis-muconic acid, which was decomposed to carbon dioxide, ammonia, and water. Although he tried to extract the enzymes responsible for such a metabolic pathway, the

Fig. 1. Osamu Hayaishi (1920–2015) at Osaka Bioscience Institute with Don Quixote.
only isolable enzyme was the one catalyzing the cleavage of the benzene ring of catechol to produce cis,cis-muconic acid (Fig. 2A). The enzyme consumed a stoichiometric amount of O2, but the oxygen molecule could not be replaced by any other hydrogen acceptor molecules known at that time. Thus, the enzyme seemed to be different from classical oxidases and dehydrogenases, and he referred to the enzyme as pyrocatechase (9). It took him five more years before he ultimately characterized this enzyme as an oxygenase.

Upon receiving an invitation letter from D. E. Green in 1949, Hayaishi traveled to Madison, WI, where he worked at the Enzyme Research Institute for several months. At the meeting of the Federation of the American Society of Experimental Biology, he attended a lecture by Arthur Kornberg, whose presentation so impressed and inspired Hayaishi that he applied for a job in Kornberg’s laboratory. Waiting for the available fellowship, he spent three productive months with Roger Stanier in California working on the bacterial metabolism of tryptophan, and then moved to the National Institutes of Health to work with Kornberg. Hayaishi subsequently accompanied Kornberg to Washington University in St. Louis and stayed there as an assistant professor with Paul Berg. What Hayaishi experienced and learned in the Kornberg laboratory established his style as a researcher and a teacher and was very influential later to a number of his students.

In 1954, Hayaishi was appointed chief of the Toxicology Section of the National Institute of Arthritis and Metabolic Diseases at the NIH, and he established his independent laboratory. To start his research program, he decided to characterize pyrocatechase enzyme, “his first love” (4). The enzyme utilized one mole of oxygen per mole of catechol. Using a heavy isotope of oxygen (18O2) generated by electrolysis of H218O, donated by D. Samuel of the Weizmann Institute in Israel, Hayaishi performed isotope labeling experiments and mass spectrometry in collaboration with M. Katagiri and S. Rothberg. Their experimental results established that two 18O atoms were incorporated into cis,cis-muconic acid from 18O2 but not from H218O (10) (Fig. 2A).

In the same year H. S. Mason and his coworkers used isotope labeling experiments with 18O2; moreover, the initiation of the lipoxygenase reaction by a regio- and stereo-specific hydrogen abstraction was demonstrated (15). Various prostatic groups and coenzymes were found in oxygenases: heme, nonheme-iron, copper, flavin, pterin, and so on (14). An important development was the discovery of a new hemoprotein named P450 (16), and its functions in steroidogenesis were elucidated by action spectra (17). The cytochrome P450s (CYP) are now known to be a large family of hemoproteins that function as monooxygenases in the biosynthesis and degradation of a variety of compounds including steroids, fatty acids, and xenobiotics.

After these prominent achievements during his eight-year stay in the United States, Hayaishi returned to Japan as a professor of medical chemistry at Kyoto University, Faculty of Medicine in 1957. He established his new laboratory not only with startup grants from the Japanese government but also with support from various American foundations including the NIH. Hayaishi was subsequently appointed a professor of biochemistry at Osaka University School of Medicine (1961–1963) and a professor of nutrition at the University of Tokyo, Faculty of Medicine (1970–1974). He began performing two lines of research, one on tryptophan metabolism and the other focused on oxygenases.

Tryptophan metabolism and oxygenases. As mentioned above, studies on the bacterial metabolism of tryptophan marked the starting point of his research career. Now Hayaishi became interested in the mammalian metabolism of tryptophan elucidating the biosynthetic pathway of nicotinic amide dinucleotide (NAD) and the structure and function of poly-ADP ribose. He and his coworkers examined the enzymatic basis for conversion of tryptophan to NAD in mammalian liver and found that 3-hydroxyanthranilic acid is enzymatically converted to niacin ribonucleotide through quinolinic acid in the presence of 5-phosphoribosyl-1-pyrophosphate (18). They extended their NAD studies by characterizing niacin phosphoribosyltransferase thereby enriching their background and resources in NAD metabolism (Fig. 3).
Inspired by the pioneering work of Paul Mandel in Strasbourg and Takashi Sugimura in Tokyo (19), Hayaishi’s group incubated variously labeled forms of NAD with rat liver nuclei and discovered that the ADP-ribose moiety of NAD was incorporated into acid insoluble fraction by polymerization, and this polymer became associated with nuclear protein. Their findings marked the discovery of poly-ADP ribose [or poly(ADP-ribose)] (20), a discovery shared by the concurrent identification of the structure by the Mandel and Sugimura groups (21, 22). Today poly-ADP-ribose is known to participate in DNA repair, apoptosis, and chromatin stabilization. The clinical effectiveness of inhibitors of poly-ADP-ribose polymerase is being tested in certain types of cancer.

In addition to the discovery of poly-ADP-ribose, the Hayaishi group also discovered a bacterial toxin-catalyzed ADP-ribosylation reaction. In 1964, Collier and Pappenheimer reported that NAD is required for inhibiting protein synthesis in a cell-free system by diphtheria toxin (23). Hayaishi and coworkers utilized their expertise in NAD biochemistry to examine this molecular mechanism and discovered that diphtheria toxin catalyzed the ADP-ribosylation of aminoacyl transferase 2 to inhibit protein synthesis (24). This was the first demonstration that a bacterial toxin is an enzyme. Subsequently, toxins derived from *cholera*, *botulinum*, and *pertussis* have been shown to catalyze similar ADP-ribosylation of various G-proteins and these toxin enzymes have been used extensively in characterizing cellular signal transduction.

The oxygenase studies began in parallel by groups in both Kyoto and Osaka attempting to purify pyrocatechase (catechol 1,2-dioxygenase) and metapyrocatechase (catechol 2,3-dioxygenase) both from pseudomonad origin. These dioxygenases were identified as nonheme iron proteins and extensively studied in terms of the role of iron. Based on various findings including substrate-binding and UV-VIS and EPR spectra, an iron-oxygen-substrate ternary complex was proposed (25). Tryptophan pyrrolase is a hemoprotein dioxygenase, and the purified enzyme from pseudomonas was subjected to spectrophotometric studies that detected unique species attributable to the ternary complex of oxygen-tryptophan-hemoprotein (26).

Salicylate hydroxylase was a monooxygenase isolated from a soil pseudomonad, and the enzyme was purified to homogeneity. The purified enzyme required flavin adenine dinucleotide (FAD) and NADH (a reduced form of NAD) for the decarboxylative hydroxylation of salicylic acid to catechol. Two other monooxygenases, lysine monooxygenase and imidazoleacetate hydroxylase, were also crystallized and identified as flavoproteins (25). A unique catalytic property of lysine monooxygenase is that the enzyme can function as an oxidase just like flavoprotein D-amino acid oxidase rather than an oxygenase depending on the carbon chain length of substrate (27). Although not directly related to the oxygenase studies, Hayaishi and colleagues extensively examined enzymes involved in amino acid metabolism such as tryptophan side chain oxidase and threonine deaminase (threonine ammonia-lyase).

International symposia on oxygenases were held in Japan on five different occasions. The first meeting was organized by K. Bloch and O. Hayaishi as a US-Japan Symposium in Kyoto in 1966. Four more symposia were organized by Hayaishi’s students, at Hakone in 1981 and in Kyoto in 1990, 2000, and 2006.

Following the enzymological studies of bacterial oxygenases, research by the Hayaishi group went on to examine mammalian oxygenases with physiological and pathological functions. Another tryptophan pyrrolase was isolated and purified from rabbit intestine. This enzyme was also a hemoprotein, which was active with both L- and D-tryptophan. The enzyme was referred to as indoleamine dioxygenase (IDO) and subjected to extensive studies (28, 29). Later, it was found that IDO could be induced by virus infection or interferon (30) and this induction resulted in depletion of tryptophan. The induction was presumed to lead to inhibition of the growth of viruses and tumors, which is now known as a major mechanism of immune suppression.

**Cyclooxygenase and prostaglandins.** Until the early 1970s, the Hayaishi group had been studying water-soluble enzymes and hydrophilic substrates, but then began a long foray into lipid biochemistry, working with enzymes involved in prostaglandin (PG) biosynthesis. In the early days, PG biosynthesis was studied using microsomal fractions of ovine vesicular gland, which were incubated with arachidonic acid. The major detectable product was PGE$_2$. In solubilizing the enzyme(s) from the microsomes of bovine vesicular gland, two enzyme fractions I and II were obtained. PGE$_2$ was produced from 8,11,14-eicosatrienoic acid in the presence of both fractions I and II. However, the product from eicosatrienoic acid incubated with fraction I was inactive with fraction II (31). In 1973, two papers reported the isolation of endoperoxide-type PGs (PGG and PGH) (32, 33).
fractions I and II were tested with these PG endoperoxides, fraction I produced PGH\(_1\) from eicosatrienoic acid in the presence of hematin and tryptophan, and Fraction II converted PGH\(_1\) to PGE\(_1\) in the presence of glutathione (31). Further purification of fraction I was a matter of trial and error, but finally the active purified enzyme appeared around pH 7.0 upon isoelectrofocusing (34). The purified enzyme catalyzed the bis-oxygenation of eicosatrienoic acid to PGG\(_1\) and this reaction required hematin. When the same enzyme was incubated with PGG\(_1\) in the presence of hematin and tryptophan, the hydroperoxide of PGG\(_1\) was reduced and PGH\(_1\) was produced. Available experimental findings indicated the enzyme had dual functions, namely, PGG synthesis and its conversion to PGH. Thus, as shown in Fig. 4, the enzyme was designated as PG endoperoxide synthase with fatty acid cyclooxygenase activity and PG hydroperoxidase activity (34). Independently, two groups purified PG endoperoxide synthase (PGH synthase, or generally referred to merely as cyclooxygenase) from sheep vesicular gland (35, 36). Availability of PG endoperoxides allowed the Hayaishi laboratory to investigate various isomerases producing PGE, PGD, PGI, and thromboxane A (37) (Fig. 4). Based on Hayaishi’s strong suggestion and discussion with Sune Bergstöm and Bengt Samuelsson, Ono Pharmaceutical Co. in Osaka (President Yuzo Ono) launched a program to develop clinical applications of PGs in 1966. The first prostaglandin used in clinical practice, PGF\(_2\)\(_\alpha\), appeared on the market in 1974 as a labor-inducing drug.

In March 1983, Hayaishi retired from Kyoto University, giving his last lecture, entitled “Every failure is a stepping-stone to success”. He was appointed president of Osaka Medical College and continued his research, focusing on the physiology of brain PGD\(_2\) with the aid of a large grant (ERATO) from the Japanese government. In 1987, the Osaka Bioscience Institute was founded with Hayaishi as its director.

Studies on PGs related to sleep and wake cycles were initiated a few years before Hayaishi’s retirement from Kyoto University, when PGD synthase and PGD\(_2\) were found enriched in the brain. The Hayaishi group further explored the physiological actions of PGD\(_2\) in the brain by microinjection of this PG into rat brain and unexpectedly found that administered animals fall asleep (38, 39). Using electroencephalography and electromyography, they determined that PGD\(_2\) induced both NonREM (NREM) and REM sleep in a pattern indistinguishable from physiological sleep in both rats and rhesus monkeys.

The PGD synthase responsible for PGD\(_2\) production in the brain was purified (40) and found to be identical to βtrace, a member of the lipocalin family and a major protein in cerebrospinal fluid. Interestingly, both this lipocalin-type PGD synthase (L-PGDS) and the DP\(_1\) type of PGD receptor mediating the sleep-inducing action of PGD\(_2\) are expressed in the leptomeninges surrounding the brain parenchyma (41). This was consistent with data that infusion of PGD\(_2\) into the subarachnoid space was most effective in sleep induction. Their subsequent works [reviewed in 42] indicated that PGD\(_2\) acting on DP\(_1\) in the arachnoid membrane released adenosine into the extracellular space that then acted on the A\(_{2A}\) adenosine receptor to induce sleep partly through GABA-mediated inhibition of the release of histamine, an arousal substance, in the hypothalamus. This sleep-inducing action of PGD\(_2\) has been suggested to be involved in deep-sleep episodes associated with systemic mastocytosis, an African sleeping sickness caused by infection with Trypanosoma, and IL-1β-induced NREM sleep. Although pharmacological manipulation of PGD synthase and DP receptor induced significant suppression of physiological sleep, the physiological sleep pattern was little affected by gene disruption of receptors putatively involved in sleep regulation, including DP\(_1\), A\(_{2A}\), and H\(_1\). These findings indicated that there are complicated redundant and compensatory mechanisms of sleep regulation. On the other hand, mice disrupted of L-PGDS gene exhibits impairment of NREM sleep rebound on sleep deprivation. Sleep was a little explored research field, and the enigma
of its regulation intrigued Hayaishi until only two years before his death (5, 42).

Not surprisingly, Hayaishi was honored with numerous awards. These included being named a foreign associate of the National Academy of Sciences of the United States (1972). He also received the Order of Culture, Japan (1972), honorary doctorates from the University of Michigan (1980) and the Karolinska Institutet (1985), and The Wolfe Foundation Prize in Israel (1986). He was named an Emeritus Member of the American Chemical Society (1995). Hayaishi directly supervised and trained almost 600 researchers in his research programs at Kyoto University, Osaka University, the University of Tokyo, and at the Osaka Bioscience Institute. More than 100 professors or directors of biochemistry were cultivated in what became known as the “Hayaishi School”. He was an outstanding researcher and a tireless teacher for us, visiting our laboratory every day and independent idea comes from your own experiments and observing nature”. He invited students to his office and wrote papers with them together face to face. He was naturally instructive, not only in performing experiments but also in his effective presentation of results for an audience with broad backgrounds. On occasions when students lost in scientific competition or were disappointed by experimental failures, Hayaishi wrote on the blackboard in English “Today is the first day for the rest of your life”.

Besides research, Hayaishi enjoyed golf, wine, and French and Italian cuisine. It was our privilege to have known him and to have shared scientific time with him.

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