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Review

Professor Howard Mason and oxygen activation

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Abstract

Our understanding of the classification, function, mechanism, and structure of the enzymes which incorporate atoms of oxygen from atmospheric molecular oxygen during catalysis is based on the thoughtful and technically challenging experiments of two giants in the field of Biochemistry, Howard Mason and Osamu Hayaishi. This volume celebrates the 50th anniversary of the discovery and characterization of these "oxygenase" enzymes and provides a broad view of how far this area of research has advanced. Professor Hayaishi describes herein his perspective on the background and major discoveries which led to the development of this field. Regrettably Howard Mason passed away at age 88 in 2003. I am indeed fortunate to have been a Ph.D. student with Howard and to have the opportunity to briefly review his role in the development of this field for this special commemorative issue of BBRC.

Keywords: Oxygen activation; Oxidases; Oxygen transferases; Mixed-function oxidases; Electron transfer oxidases

Background

Howard Stanley Mason was born in Melrose, Massachusetts, on August 20, 1914. His parents had emigrated from what is now Lithuania and were married in Lewiston, Maine, in 1913. They had one child, a son they named Howard. Following public education in Brookline, Massachusetts, Howard entered M.I.T. receiving his S.B. degree in Chemistry and Biology in 1935. After receiving his S.M. degree in 1936, he taught grammar school arithmetic, geography, and high school chemistry at the American Ruston Academy in Havana, Cuba. Returning to M.I.T. in 1938 he became a Ph.D. student in the laboratory of Professor A. Milas, a synthetic organic chemist studying oxidation mechanisms. His dissertation addressed oxidations catalyzed by the osmium tetroxide–H_2O_2 system. He then went across town to Harvard to carry out postdoctoral work (1939–1940) with Professor Arthur Michael who discovered the Michael condensation reaction.

In 1940, Howard Mason joined the Division of Industrial Hygiene at NIH where, following the outbreak of World War II, he worked on military related projects including detection of and protection against mustard gas. It was during this time that he began to study enzymatic oxidation of phenols including his initial studies on tyrosinase. He published papers on active site structure, catalytic intermediates, mechanisms of mixed-function oxidation, and dehydrogenase activities during melanin formation from tyrosine and dopa (the Raper–Mason scheme for melanin formation). As a result of this latter work, the International Pigment Cell Society awarded Howard its Gordon Medal. In 1948, he began an NCI fellowship at Cambridge, England, in the laboratory of Professor A.R. Todd. While he worked with Todd on nucleotide chemistry, Howard encountered Professor David Keilin whom he has stated "gave me a strong sense of the history of respiratory biology which dignified my own work on oxygen biochemistry." His interaction with Keilin clearly influenced his future work. The information above is edited from *curriculum vitae* notes provided by Howard to Professor Ronald Estabrook in 1994.

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Early academic career and oxygen activation

Howard was attracted to the University of Oregon Medical School in 1952. Dr. Hayaishi notes [1] that in 1955, his laboratory and that of Mason independently and concurrently characterized enzyme reactions in which O_2 is added to organic substrates. Both laboratories used $^{18}O_2$ as a tracer. Mason et al. [2] demonstrated the oxidation of dimethylphenol to dimethylcatechol by the phenolase complex and reported that the incorporated oxygen atom came from atmospheric O_2 and not H_2O. Hayaishi et al. [3] found two atoms of O_2 inserted into catechol by pyrocatechase, both coming from atmospheric oxygen.

In 1957, Mason summarized three broad classes of enzymes which activate oxygen in different ways [4,5]. First of these are a special class of oxidases (dioxygenases, aka oxygen transferases) which catalyze the consumption of one molecule of O_2 per molecule substrate with both atoms of oxygen being incorporated into the substrate. These enzymes are activated by reducing equivalents and most or all contain metal ions. Proposed oxygen activation by this class includes: (S is substrate—an organic molecule to which oxygen is incorporated).

$$S + O_2 \rightarrow SO_2$$

$$2S + O_2 \rightarrow 2SO$$

The second group are mixed-function oxidases (monooxygenases) which consume one molecule of atmospheric oxygen per molecule of substrate oxidized but only one atom of molecular O_2 is found in the product. The other atom of O_2 undergoes two-electron reduction, often being found in water. These enzymes are also generally metal containing and require reducing equivalents, often from reduced pyridine nucleotides. Oxygen activation by this class includes:

$$SH + O_2 + 2e^- \rightarrow SOH + O^{2-}(H_2O)$$

Mason noted that these enzymes catalyze both the transfer and reduction of oxygen.

The third class of oxygen activating enzymes are electron transfer oxidases which reduce molecular oxygen to hydrogen peroxide or to water. This class of enzymes catalyze two-electron transfer to O_2, forming H_2O_2 or four electrons to O_2 forming two molecules of water. Almost every enzyme able to reduce oxygen to hydrogen peroxide has an alternative, preferred electron acceptor which is frequently a coenzyme.

$$O_2 + 2e^- \rightarrow {O_2}^{2-}(H_2O_2)$$

$$O_2 + 4e^- \rightarrow 2{O_2}^{2-}(H_2O)$$

Continued studies of oxygen activation

For 40 years at the University of Oregon Medical School (now University of Oregon Health and Science University) in Portland, the Mason laboratory carried out experiments aimed at better understanding oxidases, both their mechanisms and function. One constant through these years was the international composition of his laboratory. Howard trained scientists from around the world, more from Japan than any other country. It was a laboratory of exceptional communication and hard work. For a Ph.D. student paid at the going NIH rate of $2300/year there was free food available from the enzyme sources, tyrosinase from mushrooms, P450 from New Zealand white rabbits, and best of all, hemocyanin from Dungeness crab. Lab lunches were almost always at the Tuck Lung Chinese Grocery, a particular favorite of Howard. Every afternoon at 3 PM, tea was served in the lab. The dishwasher for many years, Norah Poore, was from the U.K. and shared with Howard a passion for tea. Tea and cookies were a time for all lab members to gather together with Howard and discuss science, politics, and local activities. My path to becoming a Mason trainee was strictly by chance. I was offered a Ph.D. student position in the graduate program only if I joined the Mason laboratory. What luck! Howard was a very demanding advisor, not just for an immature student like me, but also to his most experienced fellows. The standards were very high and criticism came easy. But the rewards were high in return. We were surrounded with pioneering research on important scientific problems and very gifted colleagues. We met leaders in the study of oxidases from around the world. Whether experienced investigators on sabbatical, postdoctoral fellows or graduate students, we left the laboratory much better scientists. The most important lesson that I took with me was that control experiments are as important as the data collecting ones. Being a student with Howard Mason was an exhilarating roller coaster ride without which I doubt that I would be in science today. The demands for hard work and accuracy, and sometimes embarrassing criticism were followed infrequently by the Mason grin, a hand on the shoulder, and genuine congratulations on a good experiment.

The other constant in the Mason lab was the very high quality of data produced. Oxidases of particular interest to Howard over these years were tyrosinase, cytochrome oxidase, cytochrome P450, and NADPH cytochrome P450 reductase. Since many of these enzymes are metal-containing and free radicals were predicted intermediates in certain reactions including melanin formation, Howard Mason became very much interested in the new technology of electron spin resonance (ESR). His laboratory would use this technology extensively in many studies of oxidases in the 1960s and seventies. In 1962, he published a paper on ESR study of electron transport in rabbit liver microsomes where he first described microsomal Fex which he predicted to be a hemoprotein which could be reduced in microsomes by NADPH [6]. Earlier that year Omura and Sato [7] had reported optical studies of a new cytochrome in rabbit liver microsomes which they named cytochrome P450. It also could be reduced by NADPH. In the last paragraph of

[6], Howard wonders whether microsomal Fex is the same as the CO-binding P450. Before long it would be well established that microsomal Fex and P450 were the same hemoprotein, an important mixed function oxidase. What is most remarkable is that we now know that there are more than 5000 P450s in biology, examples being found in all kingdoms of life. Many more will be found through genomic analyses in years to come. Examples of several are presented in this volume, but Howard Mason and Osamu Hayaishi could never have anticipated the large number of mixed-function oxidases (monooxygenases) which exist throughout biology. With his colleague, Takashi Iyanagi, Howard established in 1973 that NADPH cytochrome P450 reductase, the microsomal flavoprotein that supports the activity of microsomal P450s, contains one molecule of FMN and one of FAD, rather than the two molecules of FAD as previously thought [8]. This discovery has profoundly influenced our understanding of

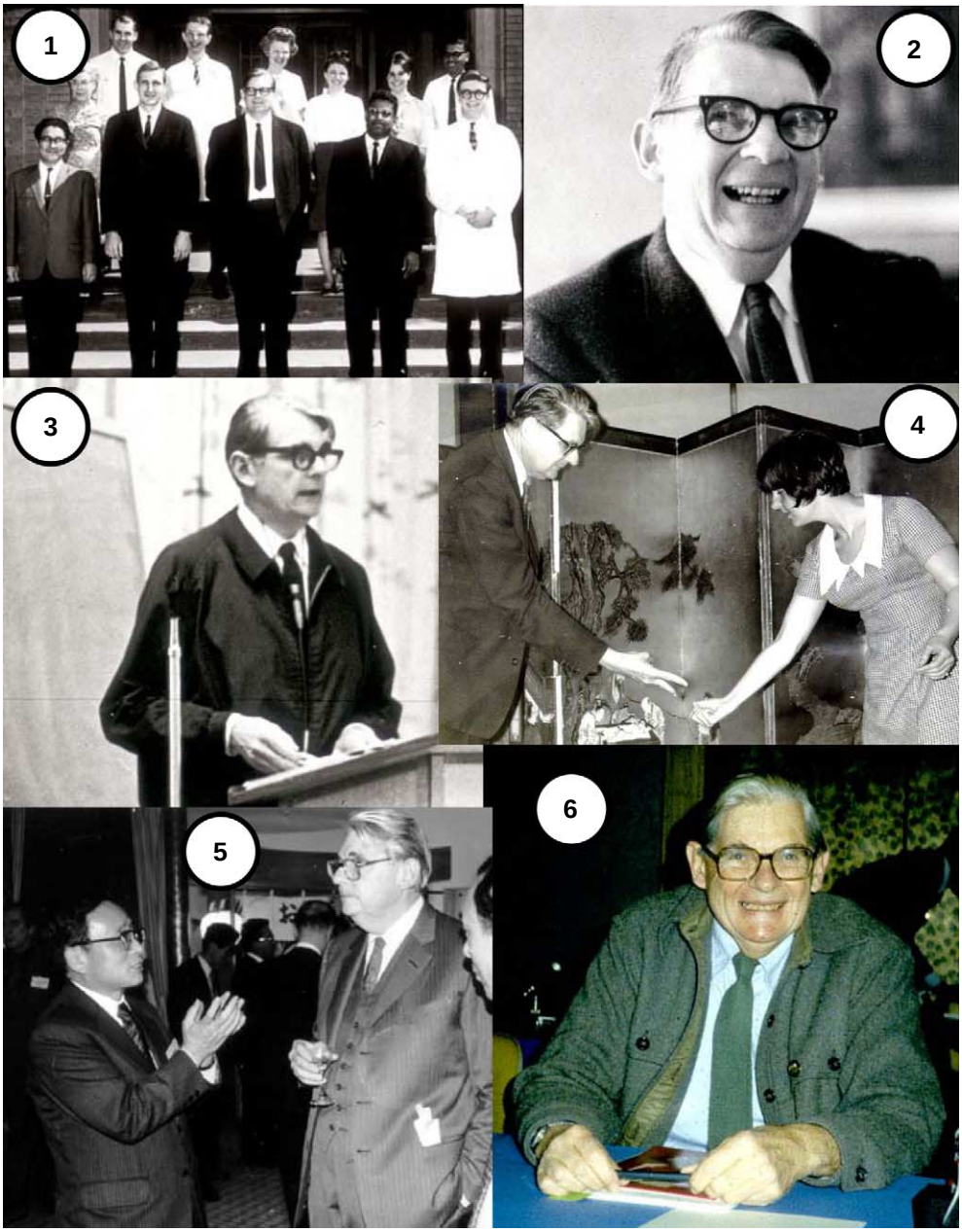

Fig. 1. Picture 1: Howard Mason (center of front row) with members of his laboratory in 1964. Note: Mike Waterman, P.M. Nair, Jim Gaylor, and Yoshihiro Miyake (right to left in the front row). Picture 2. Taken in 1964 during the NIH Biochemistry Study Section Review Committee meeting in Bethesda, MD. Picture 3. Howard Mason opening the First International Symposium on Oxidases and Related Redox Systems, Amherst, MA, July, 1964. Picture 4. Howard Mason plays "Rocks, Scissors and Paper" with his daughter, Elizabeth, at the banquet of the United States-Japan Symposium on Oxygenases, Kyoto, Japan, May, 1966. Picture 5. Toshio Yamano describes his recent results to Howard Mason at the Opening Reception of the International Symposium on Oxygenases and Oxygen Metabolism, Hakone, Japan, November, 1981. Picture 6. Howard Mason enjoys the lectures at the Second International Symposium on Oxygenases and Oxygen Metabolism, Hakone, Japan, November, 1981.

electron transfer from NADPH to cytochrome P450 and other physiological acceptors.

Howard Mason was a true scholar, and in addition to his thoughtful views on oxidases supported by extensive experimental data, he left us with other treasures. These include volumes from the four International Symposia on Oxidases and related Redox Systems which he organized with Professors Tsoo King and Martin Morrison. In addition, with Professor Marcel Florkin he crafted seven volumes of Comparative Biochemistry.

I am sure it has crossed the minds of many who knew Howard Mason, why was he not more highly recognized with honors and membership in learned societies. It may be because he was both shy and humble in his interactions with others. Howard was the master of his laboratory without a doubt. But outside that environment he generally took a step back from others, at least until he knew them well. Reflection on his accomplishments surely indicates how important Howard's work was in respiratory biology and biochemistry in general. His personality simply did

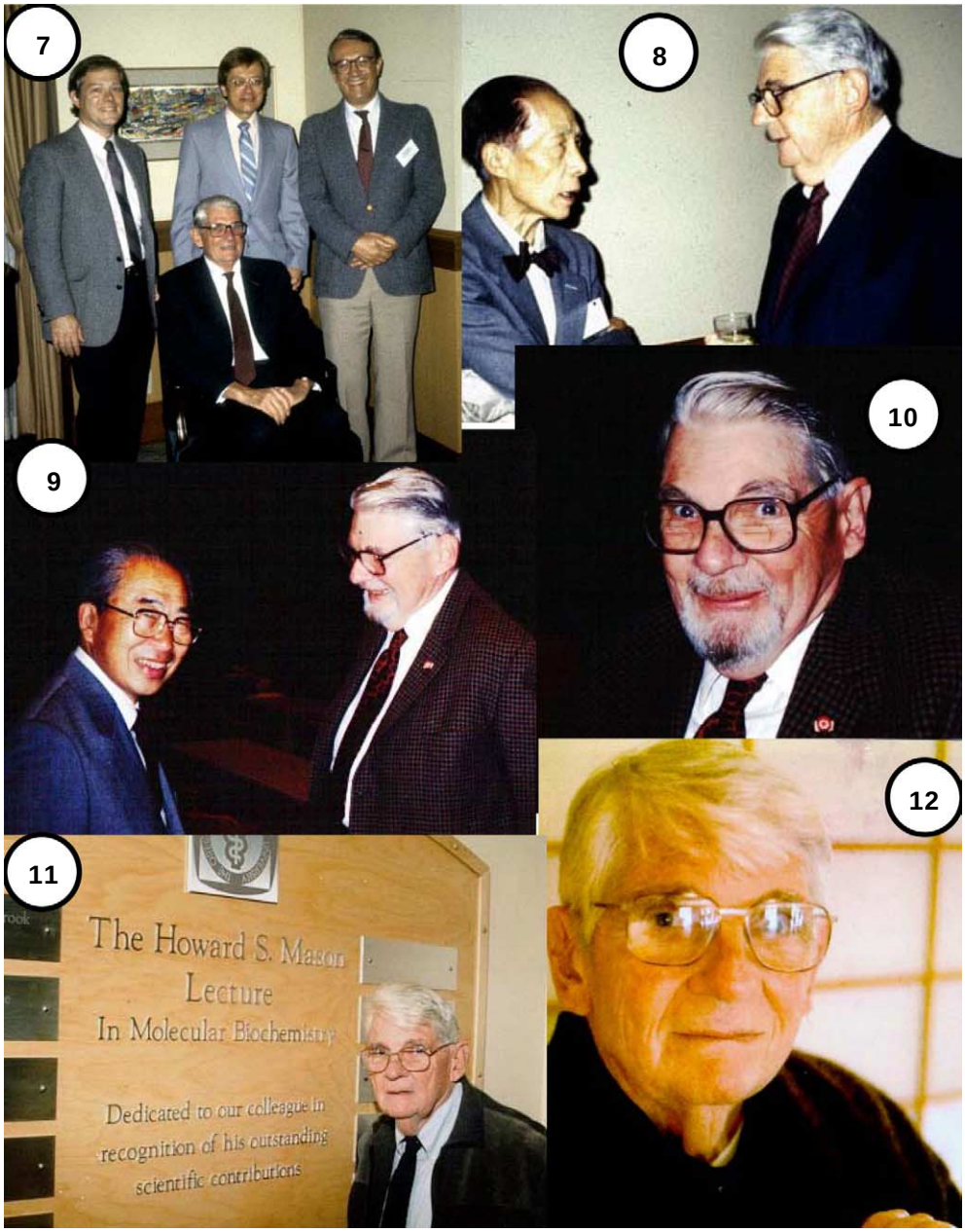

Fig. 2. Picture 7. Howard Mason poses with his former students: Dean Jones, Dan Nebert, and Mike Waterman, during the meeting of ISOX III held in Portland, Oregon, October, 1987. Picture 8. Tsoo King and Howard Mason discuss the success of the ISOX meeting held in Portland in 1987. Picture 9. Howard Mason and Osamu Hayaishi pose for a picture at the Yamada Conference "International Symposium on Oxygenases and Oxygen Activation," Kyoto, Japan, December, 1990. Picture 10. Howard Mason at the "International Symposium on Oxygenases and Oxygen Activation," Kyoto, Japan, December, 1990. Picture 11 (1995). Howard Mason poses by the plaque honoring The First Howard S. Mason Lecturer in Molecular Biochemistry, University of Oregon Health Science Center, Portland, OR. Picture 12. A recent (2001) picture of Howard Mason in retirement.

not lend itself to the recognition received by many of his peers.

Howard Mason was a devoted husband and father who shared the hobby of growing Japanese plants with his wife Margaret in the garden of their home. Another hobby was collecting Japanese stamps. Besides science, he and Ron Estabrook could talk for hours about their respective collections. While a demanding and often stern mentor, Howard enjoyed practical jokes which most often caught his colleagues very much off guard.

In closing it is important to recall that our field of oxygen activation and catalysis by enzymes has arisen from the independent yet confirming research of two such exceptional scientists as Hayaishi and Mason. Each laboratory has readily recognized the simultaneous accomplishments of the other. We are certainly fortunate at this 50-year milestone to still be led by Professor Hayaishi. However, it is also important to remember the many contributions of Howard Mason in building this field of investigation. I have chosen a quote from his *curriculum vitae* notes to provide insight into the man, both for those who knew him and those who did not.

''Looking at enzymic oxygenation broadly and trying to assess the consequences of the original breakthrough made by Professor Hayaishi and myself and our co-workers, I think it is fair to say that we discovered new large categories of enzymes involved in oxygen metabolism by routes hitherto unsuspected—direct insertion of oxygen atoms into substrates. This discovery has become important in understanding many fundamental structure—function aspects of enzymology related to oxygen and primary and secondary metabolism, regulation of gene-expression and function, and the study of the genes which code for oxidases and oxygenases, biochemical defenses against xenobiotics, oxygen toxicity in terms of partial reduction of O_2 to very reactive and toxic products, the involvement of oxygen in fundamental processes of regulation at the molecular and physiological levels of organization, the biosynthesis of hormones, the molecular biology, comparative biochemistry, and evolution of oxygen-reactive enzymes.

Altogether, and in brief: small roots, giant tree: the discovery of heavy oxygen and its application to properties of oxidases and oxygenases have opened the respiratory phenomenon to a great variety and wealth of understanding and utility.''

Acknowledgments

The author greatly appreciates the support and information provided by Ron Estabrook, who was a great friend of Howard Mason. Howard encouraged the author to take postdoctoral training with Toshio Yamano in Osaka, but disruption of Japanese Universities in the late 1960s prevented this from happening. The second recommendation was Ron who has served as an important mentor to the author for more than 35 years. Selected pictures of Howard Mason in a variety of venues during forty years are presented in Figs. 1 and 2 (provided by Ron Estabrook).

References

[1] O. Hayaishi, M. Nozaki, Nature and mechanisms of oxygenases, Science 164 (1969) 389–396.
[2] H.S. Mason, L. Fowlks, E. Peterson, Oxygen transfer and electron transport by the phenolase complex, J. Am. Chem. Soc. 77 (1955) 2851–2914.
[3] O. Hayashi, M. Katagiri, S. Rothberg, Mechanism of the pyrocatechase reaction, J. Am. Chem. Soc. 77 (1955) 5450–5451.
[4] H.S. Mason, Mechanisms of oxygen metabolism, Science 125 (1957) 1185–1188.
[5] H.S. Mason, Oxidases, Adv. Enzymol. 19 (1957) 79–223.
[6] Y. Hashimoto, T. Yamano, H.S. Mason, An electron spin resonance study of microsomal electron transport, J. Biol. Chem. 237 (1962) PC3843–PC3844.
[7] T. Omura, R. Sato, A new cytochrome in liver microsomes, J. Biol. Chem. 237 (1962) PC1375–PC1376.
[8] T. Iyanagi, H.S. Mason, Some properties of hepatic reduced nicotinamide adenine dinucleotide phosphate-cytochrome *c* reductase, Biochemistry 12 (1973) 2297–2308.