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Remembrances of Irwin C. Gunsalus—curiosity and humanity

Mitsuru Haniu*

Department of Protein Science, Amgen. Inc., One Amgen Center Dr., Thousand Oaks, CA 91320, USA

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My interest in biochemical research began with my experience at Tokyo Metropolitan University in Japan. From 1965 through 1966 I was an undergraduate student in Kazuo Satake's laboratory under the guidance of Masaru Tanaka. Dr. Tanaka had just returned from Kerry T. Yasunobu's laboratory at the University of Hawaii, where he completed the first sequencing of bacterial ferredoxin, an iron–sulfur protein. Upon attending a departmental seminar presented by M. Tanaka on the structure–function relationship of ferredoxin, I became strongly interested in those same aspects of protein molecules. I was amazed at how the protein molecules, consisting of only 20 amino acids, could have such a diversity of biological activities in such a variety of biological systems. It has been shown by scientists worldwide that the amino acid sequence determines the secondary and tertiary structures, which would consequently be important for understanding the biological functions of the proteins. However, I initially wanted to be involved in an organic chemistry laboratory for my undergraduate research since the concepts of organic chemistry are well organized and understood. Because of the limited space in the laboratory, the chairman of the Department of Chemistry asked me if I would transfer to the biological chemistry section instead. When I heard about this change, I was so disappointed about my future because I thought that biological chemistry or biochemistry research was only concerned with animal blood, tissues, chicken eggs, etc. However, after I engaged in protein structural characterization and read biochemical research references, I realized the importance of protein research, particularly in the medical field. Later M. Tanaka encouraged me to become a member of the Biochemical Society of Japan and thus my first step in discovering my interest in biochemical research was completed.

After finishing my Master of Science program in Japan from 1966 to 1968, I had an opportunity to move into K.T. Yasunobu's laboratory in 1968 with the help of a recommendation from M. Tanaka. My initial responsibility as Dr. Tanaka's assistant was to support the purification and sequence analysis of adrenodoxin from bovine adrenal tissues in collaboration with Tokuji Kimura. We experienced a great deal of challenges while working on the isolation and purification of this mammalian iron–sulfur protein. In order to obtain bovine adrenal tissues, I was required to go to the slaughterhouse near Honolulu International Airport for 3 weeks. Eventually, I could not obtain enough adrenal glands for isolation of adrenodoxin. Fortunately, K.T. Yasunobu had procured a large quantity of bovine adrenal glands from the Chicago Meat Company without any cost. Everyone involved in this project was encouraged by this great support. We had sufficient adrenal tissues to supply the needed electron transport proteins, including adrenodoxin, adrenodoxin reductase, and cytochrome P450. At that time, however, we did not have sufficient resources to study the reductase or the cytochrome P450. M. Tanaka and I worked hard day and night on the purification of adrenodoxin to yield a surplus of protein. This experience tremendously encouraged us and so we were able to proceed with the protein sequencing aspects of our research.

During my time in Yasunobu's laboratory, I saw significant technological developments in protein sequence analysis. For the sequencing of protein and peptide in the early 1970s, we primarily used a Beckman spinning cup sequencer along with manual Edman degradation. At that time, protein sequences were determined using an automated sequencer after N-terminally blocking with 4-sulfo-phenylisothiocyanate. Furthermore, PTH-amino acids had to be manually determined using a combination of TLC with amino acid analysis of the HCl hydrolyzates. It was a very primitive method compared to the modern HPLC

* Fax: 1-805-499-7464.
E-mail address: mhaniu@amgen.com.

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techniques we now use for PTH-amino acid analysis. Occasionally, John W. Crabb and George E. Tarr came to our laboratory from M.J. Coon's laboratory at the University of Michigan to work on mammalian cytochrome P450LM2. G.E. Tarr elegantly established an HPLC method for the analysis of PTH-amino acids derived from manual Edman degradation (the well-known Tarr method). He also introduced the use of a highly basic polymeric compound, Polybrene, in manual protein sequencing to tightly absorb the sample to glass vials or glass tubes. This technique is used in the ABI gas-phase sequencer even today. From this background we were very proud of Yasunobu's laboratory with respect to the advanced sequencing technology and various research projects.

In 1977, I entered the graduate program at the University of Hawaii in order to advance my research career. When I began the curriculum at the J.A. Burn's Medical School, a new organization had also been initiated as well. The Department of Biochemistry and Biophysics was a newly established department and consisted of a small group of individuals, which allowed good communication, particularly between the students and staff. During my studies, the department was so friendly and enjoyable as the staff and students helped each other like family. Since I had previously worked as a research assistant in Yasunobu's laboratory, I was able to start my research project early by choosing one of the protein sequence projects during my course work. In the laboratory there were always many research scientists and visitors from foreign countries such as Japan, Pakistan, Germany, Austria, China, Taiwan, as well as the United States. We had a departmental association time every Friday when there would be interesting seminars by prestigious guest speakers, including D. Davie, H. Neurath, A. Kornberg, R.E. Dickerson, I.C. Gunsalus, M.J. Coon, T.E. King, T. Singer, etc. We also had good collaborations in the area of electron transport proteins with such researchers as H.S. Mason, K.K. Rao, T. Kimura, D.C. Yoch, T.C. Stadman, V. Massey, and S.G. Mayhew. Since the laboratory was filled with motivated and competent people, research was routinely productive and fruitful. K.T. Yasunobu always had many interesting research projects involving a number of metalloenzymes and the related proteins, including plasma amine oxidase, monoamine oxidase, lysyl oxidase, rubredoxin, ferredoxin, flavodoxin, bioactive peptide hormone, tyrosinase, tropoelastin, cytochrome *c* oxidase, cytochrome P450, etc. From this research, K.T. Yasunobu organized the scientific conference called Frontiers in Protein Chemistry in 1979 and had invited both Asian and US scientists to the University of Hawaii. It was a precious time for all the young scientists and graduate students, including me, to hear the research reports from many prestigious scientists.

At the beginning of my graduate study, I was personally introduced to Irwin C. Gunsalus (known as "Gunny") by K.T. Yasunobu and was supported by him and his colleagues in the area of protein research where my Ph.D. thesis was the "Determination of the Amino Acid Sequence of Cytochrome P-450 from *Pseudomonas putida*." This project was shared with Gene L. Armes, a graduate student, since the protein was too big to be completed by one person. Before this project started, M. Tanaka and I had been engaged in studying the sequence analysis of putidaredoxin, an iron–sulfur protein [1], and these bacterial proteins had been provided by Gunny's laboratory. Since these proteins were purified mainly in his laboratory at that time, the collaboration proved to be very productive and enjoyable at this early stage of my protein chemistry research during the 1970s. As I reflect back on those years, I am reminded of Gunny's attitude and philosophy in his research.

Gunny expressed a strong interest in the area of technology development and its application to protein research. In 1978, a new computer system had been installed at the University of Hawaii, and Gunny was adamant about us using this sophisticated system for electronic communication between the University of Hawaii and the University of Illinois, where he was located. I was not familiar with this new e-mail system at the time and consequently lost information during several trial communications. Although Gunny must have been disappointed, he never expressed any anger over it. He was always so keen to set up any collaboration with researchers in the field of protein science, if it was applicable to his proteins. Gunny wanted to exhaust all resources to fully understand the unique characteristics of cytochrome P450 purified in his laboratory. This was reflected by Gunny's interest in the crystal structure determination of this cytochrome accomplished by Thomas L. Poulos and his colleagues at the University of California, Irvine using X-ray diffraction [2].

Gunny took pride in his publications and put great effort into his articles. When he visited our laboratory, he often carried a large bag that held many unpublished manuscripts submitted by scientists worldwide for publication in numerous journals. He took major responsibility for all of the publications involving his authorship. For instance, at the same time we published the sequence of bacterial cytochrome P450 in 1982 [3,4], Fujii-Kuriyama et al. had reported the first cDNA sequence of mammalian cytochrome P450 [5]. Consequently, Gunny discovered errors in our sequence, based on the cDNA sequence [6], and he then pushed to correct the sequence in the journal [4]. He had taken immediate responsibility for this issue and I realized the strong sincerity in his research publications.

I was also very impressed by Gunny's personality and humanity. He frequently came to our laboratory after 6 p.m. to discuss work, but I wanted to go home as soon

as possible because my family would pick me up. When we offered to drop him off at his hotel in Honolulu, he always protested and stated, "Don't worry about me. I don't want to bother you. I will catch a Yellow Cab by myself." But according to our Japanese custom, we (my wife and two daughters) never permitted him to take a cab. I stubbornly persuaded him to let us drop him off at his hotel. I also fondly recall the completion of my thesis and graduation from the University of Hawaii, at which time Gunny presented me with a congratulatory card and unforgettable gifts. This encouraged me greatly, although I did not have anything to adequately repay him for his wonderful kindness. I am very grateful to Gunny, not only for his invaluable contribution to my work but also for his person.

Throughout my graduate research, Gunny was a good educator, always providing encouragement to the young scientists. Undoubtedly, throughout his career many scientists have been trained and educated in his laboratory. One day, K.T. Yasunobu told me that Gunny had talked about my research attitude, saying, "He will become a good scientist." (Even now I do not think that I am a good scientist.) When I heard his words, I was slightly embarrassed and felt accountable for fulfilling Gunny's anticipation of me. I felt that I could not accomplish this expectation until today; however, it was his words that always encouraged me and motivated me in my later research life. For this reason I could continue my protein chemistry research to this day. One day while discussing co-authorship of a manuscript, Gunny taught us that research evaluation for individual participants should always be fair according to their contribution and this was powerful motivation for me and the other young scientists.

After I graduated from the University of Hawaii in 1981, I accepted a postdoctoral position in John E. Shively's laboratory (Division of Immunology) at the Beckman Research Institute of the City of Hope, Duarte, California. At the time, protein microsequencing was a prevailing technology in the world and it allowed me to continue the structural analysis of cytochrome P450, but of mammalian origin. Gunny said that I chose a good laboratory and a good supervisor since he knew John E. Shively very well. John E. Shively had graduated from the Department of Biochemistry at the University of Illinois, where Gunny was chairman of the department and good friends with Charles Todd, chairman of the Division of Immunology at the Beckman Research Institute of the City of Hope. I had no contact with Gunny when applying to Jack Shively's laboratory, but Gunny was already a well-known person and was respected on the west coast. I felt so insignificant in this field but yet a part of the family at the City of Hope and so I enjoyed the protein research there with Jack Shively's colleagues. In 1990, I accepted a job at Amgen, Inc., Thousand Oaks, California, where I have continued in the field of protein structure applied to therapeutic drugs. Even at that time, Gunny had helped me by introducing me to a scientist at Amgen who graduated from the University of Illinois.

Although I have had no research collaboration with Gunny since 1982, he allowed me to contact him when he founded the University of Illinois Research Fund for young scientists and professors. I am so fortunate to have worked throughout my career with this wonderful scientist, whom we all endearingly call "Gunny." He is an unforgettable person to me and I will appreciate him for many reasons. I will always be thankful for having the great opportunity to work with Gunny, who is a wonderful role model for scientists.

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