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Gunny's enthusiastic role as faculty advisor for fledgling microbiologists at Cornell

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Gunny's influence on my scientific career started when I was an unsophisticated 16-year-old country kid just starting my freshman year at Cornell. Gunny, a young assistant in the Bacteriology Department, had been given the additional responsibility of serving as advisor for a new crop of about 20 bacteriology majors even though he was still in the process of completing work for his Ph.D. To us, he seemed a much more advanced member of the faculty and we were impressed by his enthusiasm and detailed knowledge concerning the courses we were expected to take. If we complained concerning the quality and content of a required course, he took note and recommended its replacement. Whenever I expressed a doubt concerning an 18-hour per semester credit course load in addition to my 4 hours of work per day for room and board, his answer always was, "You are a good student and you can do it." In view of Gunny's own intensive work schedule, those of the undergraduates he supervised must have seemed relatively uncomplicated. Many years later I learned from Gunny that he had started out as a farm kid brought up in South Dakota and we compared notes as to some of the strengths derived from such backgrounds. Once, he incensed my mother by telling her that he knew me before I knew how to wear shoes. Her immediate response was to the effect that I had been brought up very correctly. While we were still graduate students at the University of California in Berkeley I took my new husband, Earl Stadtman, to Ithaca to visit Gunny, who by that time was a professor in the Bacteriology Department at Cornell. During the conversation, Gunny explained to Earl how to make cell-free extracts of dried bacterial cells and, in fact, gave a demonstration. Back at Berkeley, Earl tried out this procedure successfully and this changed the whole course of his Ph.D. research problem. Thanks to Gunny, he could work with a soluble enzyme system that carried out the synthesis of fatty acids instead of using suspensions of intact cells. Additional details of Earl's first contact with Gunny are given in his article in this issue. Throughout all of the passing years we have enjoyed a wonderful friendship with Gunny and it is a great pleasure to pay tribute to this remarkable scientist. I hope, Gunny, that you will approve of my decision for the continuation of this article to present a brief overview of some of my present scientific efforts instead of continuing with personal reminiscences, although the latter is very tempting.

The direction of some of our investigations in the field of selenium biochemistry has shifted recently to the identification and characterization of selenium transferases, otherwise currently referred to as selenium delivery proteins. These proteins transfer an atomic form of selenium directly to enzyme systems that otherwise would require high toxic levels of free selenide as substrate. One of the recipients of the selenium that is transferred by these proteins is the enzyme that synthesizes selenophosphate from selenide and ATP. Studies on the mechanism of synthesis of this highly reactive, oxygen-labile selenium compound have shown that the phosphorus of the Se–P bond is derived from the gamma phosphoryl group of ATP. The energy-rich selenophosphate compound appears to serve as the universal selenium donor for synthesis of selenocysteine residues that are incorporated specifically into selenium-dependent enzymes. Earlier investigations on sulfur transferases and their emerging roles in the biosynthesis of sulfur-containing cellular constituents, such as iron sulfur centers, thiouridine in tRNAs, biotin, thiamine, and lipoic acid, serve as models for study of the transfer of selenium.

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In the sulfur pathway, cysteine lyases can liberate sulfur from cysteine and rhodanese-type enzymes can mobilize sulfur from thiosulfate. The sulfur then can be delivered either directly or via accessory proteins to the various precursors. A family of cysteine lyases isolated from *Escherichia coli* that also could utilize selenocysteine as substrate [1] were used in our preliminary studies to deliver selenium to selenophosphate synthetase [2,3]. However, the fact that both cysteine and selenocysteine served as substrate indicated that a search should be made for selenium-specific delivery proteins that could function under in vivo conditions in which micromolar levels of selenium and millimolar levels of sulfur coexist. An obligately anaerobic microorganism, *Methanococcus vannielii*, that I originally isolated from San Francisco Bay mud flats in my graduate student days grows readily in a selenium-supplemented mineral salts medium with formate as sole organic substrate. A selenocysteine-containing formate dehydrogenase is synthesized [4]. In the intervening years, we utilized *M. vannielii* as a source of other selenium-containing enzymes and selenium-modified tRNAs and thus the organism could be expected to contain significant levels of proteins specialized for selenium delivery from both inorganic and organic sources. Accordingly, I examined extracts prepared from cells cultured in media containing ^{75}Se-labeled selenite for the presence of radioactive proteins in addition to the known selenium-containing formate dehydrogenase, hydrogenase, and heterodisulfide reductase components. Selenium transfer proteins containing selenium attached to sulfur of a cysteine residue as a perselenide derivative could be expected as ^{75}Se-labeled intermediates of these proteins. This type of derivative can be reasonably stable in the absence of excess levels of free thiols or when bound in a hydrophobic region of a protein that is somewhat protected from the aqueous environment. A protein containing bound radioactive selenium was detected and significant amounts of the bound selenium were retained through several purification steps that involved adsorption and elution from DEAE-Sepharose, phenyl Sepharose, butyl Sepharose, and S-200 Sepharose column matrices. Concomitant with the gradual loss of bound selenium that occurred during these steps there was the appearance of an unlabeled protein that migrated as a 33-kDa band on SDS–PAGE gels just behind the ^{75}Se-labeled protein band. The amino acid sequences of these proteins, derived by Edman degradative analysis of extracts of protein bands cut from the gels, were identical for both labeled and unlabeled protein species. The amino acids comprising residues 1 to 63 from the amino terminus of this protein were identified and confirmed in isolates from different enzyme preparations. In spite of the fact that the determined sequence represented about 20% of the total protein, no corresponding sequence was found in any recorded data base from 1995 until 2001, when the partial genome sequence of a related methane-producing organism, *Methanococcus maripaludis*, became available. A sequence almost identical to that of residues 1–63 of the *M. vannielii* protein appeared in an open reading frame of the *M. maripaludis* genome. This was identified by the computer as an unknown 81-amino-acid protein with no known homologs. Based on this DNA sequence data, the corresponding *M. vannielii* DNA could be isolated, cloned, and expressed in *E. coli* as a selenium-binding protein. Studies on this interesting protein are in progress.

In the meantime, proteins that exhibited selenocysteine lyase activities were purified from *M. vannielii* and these were shown by partial sequence analysis to be related to members of a sulfur transferase family termed NIFS proteins. After many purification steps, I finally could separate two similar proteins—one of these exhibited cysteine lyase activity and little or no activity on selenocysteine, whereas the protein in the adjacent peak of the DEAE Sepharose profile appeared to be specific for selenocysteine as substrate. Proof that the second protein is indeed specific for the selenoamino acid substrate awaits isolation of larger amounts needed for characterization. If this proves to be the case, then it serves as an example of an enzyme that can mobilize the small amounts of selenium needed for specific selenoprotein biosynthesis in the presence of a high excess of corresponding sulfur compounds. At present, selenophosphate synthetase that converts ATP and selenide to selenophosphate is one of the few known enzymes that effectively discriminates between selenium and sulfur.

In summary, Gunny, I hope you enjoy reading this little account of my recent scientific activities in the realm of anaerobic bacterial metabolism. Certainly, things that I learned as an undergraduate in bacteriology at Cornell have served me well over all these intervening years. Thank you for being such a patient faculty advisor for a beginner in your field of scientific interest.

References

[1] H. Mihara, M. Maeda, T. Fujii, T. Kuriihara, Y. Hata, N. Esaki, A *nifS*-like gene, *csdB*, encodes an *Escherichia coli* counterpart of mammalian selenocysteine lyase: gene cloning, purification, characterization and preliminary X-ray crystallographic studies, J. Biol. Chem. 274 (1999) 14768–14772.

[2] G.M. Lacourciere, H. Mihara, T. Kurihara, N. Esaki, T.C. Stadtman, *Escherichia coli* NifS-like proteins provide selenium in the pathway for the biosynthesis of selenophosphate, J. Biol. Chem. 275 (2000) 23769–23773.

[3] G.M. Lacourciere, Selenium is mobilized in vivo from free selenocysteine and is incorporated specifically into formate dehydrogenase H and tRNA nucleosides, J. Bacteriol. 184 (2002) 1940–1946.

[4] J.B. Jones, G.L. Dilworth, T.C. Stadtman, Occurrence of selenocysteine in the selenium-dependent formate dehydrogenase of *Methanococcus vannielii*, Arch. Biochem. Biophys. 195 (1979) 255–260.