A Gold Mine of Fascinating Enzymes: Those Remarkable, Strictly Anaerobic Bacteria, Methanococcus vannielii and Clostridium sticklandii

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Anaerobic Metabolism Background

I was fortunate to have had Dr. H. A. Barker as a mentor for my graduate studies at Berkeley. He had been trained by famous microbiologists from the Delft School of Microbiology, and he instilled in his students a deep interest in the metabolism of anaerobic microorganisms. Members of the Delft School had found that the microbial decomposition of various compounds under anaerobic conditions sometimes involved unusual chemical reactions that were amenable to detailed study because the responsible catalysts were present in exaggerated amounts or subsequent rate-limiting steps allowed intermediates to accumulate. By studying under Barker, who had worked with C. B. van Niel in Pacific Grove and later with A. J. Kluyver in Delft, I became an indirect descendent of the Delft microbiologists.

For my thesis problem, I chose to work on the biosynthesis of methane, an area of research that I knew to be of considerable interest to Barker. Formate, acetate, and various fatty acids were added to simple mineral salts media for selection of organisms able to utilize these substrates for methane production. I used soil samples as inocula that I had dug from San Francisco Bay mud flats. At that time, the bay was heavily contaminated, and the mud flats reeked of hydrogen sulfide at low tide, a clear indication of anaerobic conditions. Microorganisms that grew on acetate, propionate, and short chain fatty acids were obtained, and these were studied using 14C-labeled substrates to determine the source of methane (1). Particularly interesting was the unexpected finding that in the fermentation of acetate methane was derived from the methyl carbon and the carboxyl carbon was the source of the carbon dioxide (2). Barker (Fig. 1) was particularly excited by these results because they were an exception to an earlier hypothesis of van Niel that methane is derived exclusively from carbon dioxide. However, in the other fatty acid fermentations carbon dioxide did serve as oxidant and was reduced to methane.

Formate was actively fermented to a mixture of methane, hydrogen, and carbon dioxide. Two microorganisms were enriched in parallel when formate was supplied as substrate. One proved to be a methane-producing motile coccus that we named Methanococcus vannielii in honor of C. B. van Niel (3). The other was a rod-shaped organism that I later named Clostridium sticklandii (4).

Detailed studies on the morphology and biochemical properties of M. vannielii (3) constituted a major portion of my Ph.D. thesis, and I was very gratified that the results of all of these studies were published with Barker in a series of papers on methane fermentations.

Aerobic Metabolism of Cholesterol

When I first joined the newly formed National Heart Institute at NIH in 1950, I continued working on a project initiated during the year I spent as a postdoctoral fellow at Harvard Medical School. The oxidation of cholesterol using enzymes from an aerobic Nocardia species
was investigated. At that time, sterols formed by selective oxidation of the cholesterol side chain would have been useful precursors of cortisone and certain hormones such as progesterone. Unfortunately, cholesterol was degraded completely by the organism under a variety of conditions, and we could not detect any of the desired intermediate products. However, an enzyme that oxidized cholesterol at position 3 of the ring to form Δ4-cholestene-3-one was identified and partially purified. Later, preparations of this cholesterol oxidase were produced by P-L Biochemicals, Inc. and used clinically for determination of cholesterol.

Amino Acid Fermentation Studies

After my unsuccessful experience with strictly aerobic bacteria as enzyme sources, I was glad to retreat to the anaerobic world and initiated studies on the fermentation of amino acid substrates by the clostridial species I had isolated along with *M. vannielii* from San Francisco Bay mud (4). Initial studies on the anaerobic metabolism of lysine and ornithine (5) and the roles of vitamin B₁₂ in these processes (6) provided examples of interesting new reactions and additional roles of B₁₂ coenzyme. The degradation of lysine to acetate, butyrate, and ammonia occurred by two distinct processes. In one, acetate was derived from carbon atoms 1 and 2 of the 6-carbon chain of L-lysine, and the residual 4 carbon atoms were converted to butyrate. In the other, acetate was derived from carbon atoms 5 and 6 of D-lysine and butyrate from carbon atoms 1–4. These conversions required the participation of an imposing list of cofactors and involved many distinct enzymic steps (7).

Another amino acid transformation investigated in the early studies was the reductive deamination of glycine by *C. sticklandii*. Significantly, glycine reduction proved to be an energy-conserving process linked eventually to the formation of ATP (8). Thus, in the presence of orthophosphate and ADP, glycine was reduced to acetate and ammonia and ATP was formed with the stoichiometry shown in Equation 1.

\[
\text{Glycine} + R(SH)_2 + P_i + \text{ADP} \rightarrow \text{acetate} + \text{ammonia} + R-\text{SS} + \text{ATP} \quad (\text{Eq. 1})
\]

Much later the direct products of the glycine reductase complex were shown to be ammonia and acetyl phosphate (9), and the highly active acetate kinase contaminant in the enzyme
preparations converted acetyl phosphate and ADP to acetate and ATP. The key discovery that one of the protein components of the glycine reductase complex is a selenoprotein (10, 11) shifted the emphasis of my later research.

**The Selenium Era**

Much to my surprise the studies on glycine reductase from *C. sticklandii* led us to the discovery early in May 1972 that the “rich culture medium” containing 2% Tryptone, 1% yeast extract, and formate used for routine growth of the organism was selenium-deficient. When supplemented with 1 μM selenite, the cell population exhibited high glycine reductase activity throughout the entire growth phase, whereas in the absence of added selenite glycine reductase was detected only in early log phase cells. A low molecular weight acidic protein component of the glycine reductase complex (10), termed protein A, that we had isolated previously from early log phase cells proved to be the missing factor in cell-free extracts prepared from end of log phase cell populations that were not supplemented with selenium. A typical dilution curve was exhibited for protein A levels in extracts as a function of growth of *C. sticklandii* in the non-selenite-supplemented medium. In retrospect, I realized that in the 1950s, when the Bethesda tap water could be used for culture of various anaerobic bacteria, the levels of glycine reductase in *C. sticklandii* were considerably higher than they were later when we were forced to use distilled water because of high levels of neutral detergents in the water supply. It is evident that many of the so-called “rich culture media” used by microbiologists are selenium-deficient, and this is true also for various serum-supplemented media used for culture of mammalian cells.

To determine whether selenium was an actual component of protein A, *C. sticklandii* was grown in media containing [75Se]selenite. This resulted in the incorporation of radioactivity in protein A, and the 75Se content of the protein was enriched in parallel with enzyme activity during isolation of the protein in near homogeneous form (11). Thus, by the end of June 1972 we had evidence of the existence of an essential selenium-containing protein, the protein A component of glycine reductase.

There followed a “learning period” for me concerning the chemistry of selenium and its relative, sulfur, to determine the identity of the selenium compound in the labeled protein A. I obtained several organoselenium compounds from the National Cancer Institute library that originally had been collected as potential carcinogens. However, the chemists who had synthesized these compounds had introduced phenyl groups for stability purposes, thus limiting their use as possible model compounds for our studies. Before the identification in 1957 of selenium as an essential nutrient for rats (12) and birds (13), it was known in biology mainly for its toxic properties.

**Identification of Selenocysteine in Glycine Reductase Protein A**

I had determined previously (10) that reaction of the reduced form of protein A with iodoacetamide inhibited its biological activity as an essential component of the glycine reductase complex and had assumed that one or more essential SH groups had been alkylated. When we treated the 75Se-labeled protein with iodoacetamide or iodoacetate, the biological activity likewise was destroyed, but elimination of radioactive selenium as inorganic forms previously observed during acid hydrolysis was prevented almost completely. Instead, we could recover the radiolabel from the acid hydrolysates in a compound containing an alkyl group attached to the selenium. This derivative was identified as Se-carboxymethyl-selenocysteine by comparison with the corresponding alkyl derivative of authentic selenocysteine (14). We made several other alkyl derivatives of the selenoprotein for further identification and established that the Se-carboxymethyl, Se-carboxyethyl, and Se-aminoethyl forms were the most satisfactory from the standpoint of stability during acid hydrolysis and subsequent chromatographic separation on an amino acid analyzer column. Throughout these studies, my able assistant, Joe Nathan Davis, provided invaluable expertise and together with two post-doctoral fellows, Joyce Cone and Raphael Martin del Rio, we could establish that protein A contains 1 gram atom of selenium per mol and the selenium is present in the form of a selenocysteine residue in the polypeptide (14). The methods we developed for identification of selenocysteine in our bacterial protein were used later by other investigators to isolate and identify the selenium-containing moiety in mammalian glutathione peroxidase, another enzyme that had been reported in 1973 to contain selenium (15).
To determine whether free added selenocysteine could be incorporated into protein A, Gregory Dilworth, a postdoctoral fellow in my laboratory, synthesized selenocysteine labeled either with \(^3\)H, \(^{75}\)Se, or \(^{14}\)C, and we grew \(C.\) sticklandii in the presence of these added labeled substrates. There was no detectable incorporation of the labeled carbon chain of the amino acid into protein A, but the \([^{75}\text{Se}]\)selenocysteine was used more efficiently as a selenium source than the normal supplement \([^{75}\text{Se}]\)selenite, which is reduced by thiols in the culture medium (16). In retrospect, the facile utilization of selenium from selenocysteine for protein A biosynthesis observed in these experiments is indicative of the participation of a selenocysteine lyase. These lyases, first purified by Kenji Soda and his collaborators in Kyoto from bacteria (17) and from liver (18), convert selenocysteine to an atomic form of selenium and alanine.

\[
\text{SeHCH}_2\text{CHNH}_2\text{COOH} \rightarrow \text{Se} + \text{CH}_3\text{CHNH}_2\text{COOH} \quad \text{(Eq. 2)}
\]

For several years, we thought these proteins served solely as detoxification agents, but they now are considered to function as selenium transferases or selenium delivery proteins. Much of our current research is directed to elucidation of the chemical properties and biochemical roles of these selenium transferases.

Dr. Richard Glass, a sulfur organic chemist, joined our group in 1987 while on sabbatical leave from the University of Arizona. We had met in 1984 in Lindau, Germany, at a Symposium on the Organic Chemistry of Sulfur. At this meeting the organizers had decided to enlarge the program to include biologically important selenium compounds, and my presentation on the small selenoprotein component of glycine reductase stimulated Dick Glass to become involved in selenium organic chemistry. During his year in Bethesda, we grew \(C.\) sticklandii in the presence of \(^{77}\)Se and isolated selenoprotein A labeled with the stable isotope. This was used to investigate conformational properties of the selenoprotein using \(^{77}\)Se NMR spectroscopy as a probe. Later, when we discovered that the biological donor for biosynthesis of selenuridine in tRNAs is selenophosphate (19), the synthesis of this compound was achieved by Glass and his group, and authentic selenophosphate was supplied to us as a reference compound (20). I continue to rely on Dick Glass for advice and assistance concerning a wide variety of problems we encounter in the field of selenium chemistry.

**The UGA Codon Is Used for Specific Selenocysteine Incorporation**

The presence of an unusual amino acid in two selenoenzymes, glycine reductase and glutathione peroxidase, that was not specified by the genetic code posed the problem of the method of specific incorporation of a selenoamino acid in the proteins. In fact, 13 years elapsed before it was recognized that one of the three stop codons, UGA, is used as the signal for selenocysteine insertion into a growing polypeptide chain. Simultaneously it was shown by August Böck and his collaborators in München that the TGA codon in the Escherichia coli formate dehydrogenase H gene directed selenocysteine incorporation into the protein (21) and by P. R. Harrison and his collaborators in Glasgow that the TGA codon in the murine glutathione peroxidase gene corresponded to the position of selenocysteine in bovine glutathione peroxidase (22). The amino acid sequence of bovine glutathione peroxidase had been determined earlier at Grunenthal GmbH in Aachen by Flohé and associates (23). Eventually, I could verify that selenocysteine occurred in the formate dehydrogenase protein in the position predicted by the TGA codon in the gene (24). In a series of elegant experiments by August Böck and his associates, genes were isolated that complemented some of the mutant strains of \(E.\) coli defective in synthesis of formate dehydrogenase that had been isolated previously by Marie Andre Mandrand-Berthelot (25–27). Four genes that encoded four different products essential for the specific synthesis of selenocysteine and its insertion into protein were cloned and the expressed products characterized. In one step, a serine esterified to a special tRNA (\(selC\) product, anticodon UCA complementary to UGA) is converted to selenocysteinyl-tRNA by a pyridoxal phosphate-dependent selenocysteine synthase (\(selA\) gene product) using selenium from selenophosphate, produced by selenophosphate synthetase, the \(selD\) gene product (19, 27, 28). A unique elongation factor (the \(selB\) gene product) that binds a secondary stem loop structure located 3’ to the UGA in the \(E.\) coli \(fthF\) mRNA forms a complex with the selenocysteinyl-tRNA for delivery at the ribosome site and insertion of selenocysteine at UGA (29). Refinements of these groundbreaking discoveries still are being made by many investigators in the field, particularly with respect to the differing modes of recognition of UGA for selenocysteine incorporation in eukaryotes, archae, and \(E.\) coli. This process that prevents...
operation of the usual translation termination step and instead directs insertion of selenocysteine at a specific in-frame UGA codon is an important example of a growing list of exceptions to the established stop codon rules (30).

**E. coli Formate Dehydrogenase**

David Grahame and Milton Axley, working in the anaerobic laboratory at NIH, developed an elegant two-step chromatographic procedure for isolation of the markedly oxygen-sensitive 80-kDa *E. coli* formate dehydrogenase H (31) in highly purified form. This enzyme contains molybdenum in a molybdopterin cofactor in addition to the selenocysteine in the polypeptide. Detailed kinetic analysis of the enzyme (32) and a comparison of the catalytic advantages afforded by selenium over sulfur revealed (33) that the selenocysteine-containing native or wild-type enzyme was about 300 times more active than the selenocysteine/Cys mutant for oxidation of formate with benzyl viologen as the artificial electron acceptor.

A few years later, it was shown in EPR studies that the selenium of the selenocysteine residue in formate dehydrogenase is coordinated directly to the molybdenum in the molybdopterin cofactor (34). The oxidation of formate by this enzyme does not involve a typical molybdenum-dependent hydroxylation mechanism. Instead, formate is converted directly to carbon dioxide without introduction of oxygen from solvent (35). Crystallization of the oxygen-labile enzyme under strictly anaerobic conditions was achieved (36) and based on analysis of the crystal structure (37), it was deduced that the selenium serves as the immediate proton acceptor in the reaction. This would suggest an effect of neighboring protein groups because usually at neutral pH a selenol is almost fully ionized. In contrast, from x-ray absorption spectroscopy (EXAFS) studies of oxidized and reduced forms of the enzyme, a novel seleno-sulfide ligation to the molybdenum was proposed as the proton acceptor (38). A possible alternative mechanism involving hydride or hydrogen atom transfer from formate to the selenosulfide instead of proton transfer also was suggested. Based on these somewhat differing types of evidence, the exact mechanism of action of the *E. coli* formate dehydrogenase and the precise role of selenium in the enzyme remain to be established. *In vivo*, the reducing equivalents from formate oxidation are transferred via an iron sulfur cluster eventually to a hydrogenase, and hydrogen gas is evolved.

**Selenocysteine Incorporation in Clostridia**

Despite the dearth of information concerning the genetic makeup of anaerobic spore-forming members of the genus *Clostridium*, Greg Garcia was able to isolate and clone the glycine reductase selenoprotein A gene from two different clostridia (39, 40) and establish that an in-frame TGA codon in each corresponded to selenocysteine at position 44 in the polypeptides (41). However, attempts to express the *C. sticklandii* cloned gene in *E. coli* were only partially successful (40). The full-length, 18-kDa immunologically reactive protein was produced in good yield, but the catalytic activity as a component of glycine reductase was only about 10% that of native selenoprotein. A full-length protein produced in the absence of selenium or in a SelD mutant unable to synthesize selenophosphate was inactive. Detailed analysis showed that read-through and suppression of the UGA codon involved a cysteine-tRNA, and either cysteine or occasionally selenocysteine esterified to the tRNA was inserted. It was concluded that the mRNA secondary stem-loop structure required by *E. coli* for UGA-directed specific selenocysteine insertion was not present in the clostridial mRNA structure. Although details of rules concerning clostridial selenoprotein gene expression are still lacking, the now available genomic sequence of *Clostridium difficile* should provide information on the SECIS stem-loop structure involved and its location in the mRNA. In fact, both glycine reductase and formate dehydrogenase were detected in extracts of a strain of *C. difficile* that we used in our studies in the 1950s, and the corresponding genes have been found in the published genomic structure. It is clear that the statement commonly made in the literature to the effect that in all prokaryotes the SECIS element is identical to that in *E. coli* and is located in the same orientation as in FdhF is incorrect. *E. coli* is not representative of all prokaryotes.

**Seleno-tRNAs**

Another type of biochemical process in which selenophosphate is utilized as selenium donor is the synthesis of 2-selenouridine in the “wobble position” of the anticodons of certain tRNAs. The 2-selenouridine residue in the form of 5-methylaminomethyl-2-selenouridine had been identified earlier in the lysine and glutamate tRNAs of *E. coli*, *Salmonella typhimurium*,
C. sticklandii, and M. vannielii (42). When Dr. Zsuzsanna Veres, a young Hungarian scientist from Budapest, came to my laboratory as a Visiting Fellow she decided to work on the biochemistry of 2-selenouridine. My good luck in having Dr. Veres as a collaborator came about as a result of my visit to Budapest in 1988 as a member of a USA National Academy of Sciences committee commissioned to evaluate the mutual benefits of exchanges between the United States and the Hungarian Academies of Sciences. When my counterpart on the Hungarian Academy committee, Professor Geza Denes, heard that I had an opening in my laboratory for a Foreign Visiting Scientist, he suggested his best student as a possible candidate. Dr. Veres was interested and after the usual formalities she arrived in my laboratory in August 1989. Thus started a very fruitful collaboration of almost 5 years and a deep friendship developed. During Zsuzsa’s stay in the laboratory, she demonstrated that the ATP requirement for selenouridine synthesis in tRNAs was explained entirely by its use in the generation of selenophosphate, the biological donor of selenium in the reaction (19, 20, 43). A subsequent step involved the replacement of a sulfur in the 2-thiouridine precursor in tRNA with selenium to form the 2-selenouridine residue, and the responsible enzyme system was partially purified (44). Veres’ detailed studies on selenophosphate synthetase and the role of the enzyme in the generation of the new high energy selenium donor compound were very important contributions to the overall field of selenium biochemistry. When Dr. Ick Young Kim, a young investigator from Korea, arrived, he joined Veres in studies on selenophosphate synthetase, and by site-directed mutagenesis he produced several useful mutant forms of the enzyme (45). The Veres-Kim collaboration proved to be very productive in this investigation.

**Selenophosphate Synthetase Reaction Mechanism**

Later studies in my laboratory on the selenophosphate synthetase reaction mechanism by Dr. Heidi Walker demonstrated that a group on the enzyme first is phosphorylated by reaction with ATP (46). This phosphoryl group derived from the γ-phosphoryl group of ATP subsequently is transferred to selenium to form selenophosphate. The ADP moiety of ATP, which is tightly bound during the initial step, then is cleaved in a hydrolytic step to form orthophosphate (derived from the β-phosphoryl group) and AMP (47). Earlier, we thought that a pyrophosphoryl derivative of the enzyme might be formed in the initial reaction with ATP leaving AMP as the other product. However, Song Liu’s experiments showed that retention of 32P from β-32P-labeled ATP was insignificant, whereas when γ-32P-labeled ATP was used up to 0.6 eq of 32P was bound to the enzyme (48). Details of the mechanism of the selenophosphate synthetase reaction are still under investigation.

**Selenium-dependent Enzymes That Do Not Contain Selenocysteine**

A group of molybdopterin-dependent hydroxylases, nicotinic acid hydroxylase (49, 50), xanthine dehydrogenase, and purine hydroxylase (51), that have been purified from anaerobic bacteria require selenium for activity. However, the selenium in these enzymes is not present in selenocysteine residues in the polypeptides but instead occurs in a labile cofactor. The selenium can be released from the cofactor by treatment with cyanide and thus might be in the form of a perselenide. The mechanism of incorporation of selenium in these enzymes currently is being investigated by Dr. William Self, who discovered purine hydroxylase, the most recent addition to the list of selenium-dependent hydroxylases. It was shown earlier in EPR studies that the selenium in nicotinic hydroxylase is coordinated to the molybdenum of the molybdopterin cofactor (50). In contrast to the hydroxylases from anaerobic bacteria, the corresponding enzymes from eukaryotes have not been shown to be selenoenzymes.

**Discovery of a New Mammalian Selenoenzyme**

Our studies on thioredoxin reductase were initiated as the result of a serendipitous discovery made by Dr. Takashi Tamura, a young Japanese Visiting Fellow in our laboratory, during his one-year leave of absence from Okayama University. A cytochrome P-450 present in a human lung adenocarcinoma cell line had been predicted to contain selenocysteine based on the occurrence of a TGA codon in the open reading frame of the corresponding gene (52). The possibility that the putative selenocysteine residue, located at some distance from the conserved cysteine at the heme binding site, might have a novel role in the enzyme prompted Dr. Tamura to attempt isolation of the protein. The lung adenocarcinoma cells were cultured in the presence of [75Se]selenite, and the expected 57-kDa protein labeled with 75Se was isolated in near homogeneous form. Two different alkyl derivatives of the protein were prepared, and the
corresponding alkyl [\textsuperscript{75}Se]selenocysteines were isolated and identified. The chromophore bound to the protein, however, proved to be FAD instead of a heme group. The FAD could be reduced specifically by NADPH, and various disulfides, including thioredoxin, served as substrates for the enzyme. It was evident that this selenoprotein, a homodimer of 57-kDa subunits, must be thioredoxin reductase, an enzyme that had been purified from various mammalian tissues and studied by other investigators but never suspected to be a selenoenzyme. Although the reported sequence of a putative thioredoxin reductase gene from human placenta (53) contained a TGA codon near the C terminus, this had been interpreted as a termination signal. Subsequently, experiments carried out in my laboratory by Vadim Gladyshchev (54) and by Song Liu (55) showed that the selenocysteine in thioredoxin reductase, previously identified by Tamura, occurs at the C terminus in the sequence -Cys-selenocysteine-Gly in a position corresponding to the TGA codon in the placental gene. Thioredoxin reductases purified from human Jurkat T cells (54), from HeLa cells, and from the human adenocarcinoma cells (55) were shown to have the same C-terminal triplet peptide sequences.

The importance of the potential C-terminal redox center for catalytic activity was shown in experiments with the HeLa cell enzyme by Sergey Gorlatov (56). Alkylation of the NADPH-reduced enzyme under conditions that limited alkyl group incorporation to the one ionized selenol per subunit was sufficient to inhibit catalytic activity 99% (56). When HeLa cells were grown at higher than optimal oxygen levels, the isolated enzyme consisted of significant amounts of species that contained an average of 0.5 instead of 1 selenium atom per subunit, and these forms exhibited correspondingly lowered catalytic activities (57). Reduced forms of thioredoxin reductase were very oxygen-labile in the absence of bound pyridine nucleotide, and corresponding losses of selenium and catalytic activity were observed. It now is generally agreed by a number of investigators that premature termination of gene expression at the UGA codon gives rise to a truncated inactive form of the enzyme, and mutant enzyme species in which a cysteine residue replaces the selenocysteine exhibit very low catalytic activity (58).

It thus is evident that the additional C-terminal redox center present in fully active mammalian thioredoxin reductases is an important determinant of catalytic activity and is essential in addition to the bound FAD and the redox active cysteine pair near the NADPH binding site in the N-terminal region of the protein.

Selenium Transport Proteins

One of the interesting recent developments in the selenium field comes from the realization of the importance of selenium transport or delivery proteins in selenoprotein biosynthesis. Our specific interest involves the participation of these proteins in supplying selenium for selenophosphate biosynthesis. Even under \textit{in vitro} conditions an atomic form of selenium provided by a delivery protein is used more efficiently as substrate by selenophosphate synthetase than millimolar levels of selenide normally added to reaction mixtures (59). The atomic form of selenium can be derived from free selenocysteine by selenocysteine lyases that are structurally related to the NiF sulfur transferase family of proteins (60) or from inorganic selenium compounds, \textit{i.e.} selenite after reaction with thiols to form RS-Se-SR adducts. Enzymes, such as rhodanese, that can transfer the sulfane sulfur from thiosulfate to cyanide generate a persulfide derivative of an active cysteine residue as the enzyme-bound intermediate (61). In an analogous process, a perselenide derivative of a reactive cysteine residue could be generated by reaction with a selenium substrate (62). An unusual selenium-binding protein that I isolated recently from \textit{M. vannielii} appears to be a candidate for such a role. The gene that encodes this protein was isolated, cloned, and expressed in \textit{E. coli} by Dr. William Self. Several properties of the isolated protein expressed in \textit{E. coli} are identical to those of the native \textit{M. vannielii} protein, and preliminary studies by Dr. Self suggest that the ability to bind selenium may be specific. If so, this could imply a role in supplying the significant levels of selenium required for synthesis of multiple selenoenzymes involved in the energy metabolism of \textit{M. vannielii} (63). The ability to discriminate between selenium present at micromolar concentrations in most biological systems as compared with millimolar levels of sulfur compounds is essential for efficient biosynthesis of specific selenium-containing catalysts.

The Famous NIH Anaerobic Laboratory

An anaerobic laboratory facility (Nitrogen Laboratory) that was constructed in the 1960s in Building 3 at NIH at a cost of $250,000 was used for numerous studies on oxygen-sensitive organic catalysts and oxygen-labile enzymes until March 2001 when the occupants of Building
3 were moved to Building 50. The walls of the anaerobic facility are made of 3⁄8-inch carbon steel plates that were welded into place and supported by a framework of I-beams. Floor plates were riveted to a concrete floor. All joints were welded and sealed. A nitrogen atmosphere containing hydrogen, introduced to remove the last traces of oxygen by passage through a palladium catalyst bed, was maintained at less than 10 ppm of oxygen.

This laboratory is still the only one of its kind in the world. For one of the first tests of the new anaerobic facility, I inoculated Petri dishes containing a formate-mineral salts agar medium with a culture of M. vannielii and placed them in an ordinary 37 °C incubator. When I inspected the plates the next day, I was so delighted to find colonies of this extremely oxygen-sensitive organism on the surface of the agar that I laughed, and this caused enough nitrogen to leak into my mask to set off the alarm system. I then made a rule not to laugh in the anaerobic laboratory. Seriously, over the years, we and investigators from other NIH laboratories and several universities have carried out large scale isolations of oxygen-labile enzymes and characterized oxygen-sensitive flavoproteins, B_{12} coenzyme-dependent enzymes, and selenoproteins in this anaerobic facility (64, 65). To conduct many of these procedures in an anaerobic glove box is either cumbersome or impossible as we are learning to our sorrow. Sadly, this important anaerobic laboratory facility will soon be demolished to convert Building 3 into administrative office space.

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