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Biochemical and Biophysical Research Communications 312 (2003) 41–43
BBRC
www.elsevier.com/locate/ybbrc

Enzymology is basic to an understanding of intermediary metabolism

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Received 24 September 2003

In 1947, I began my graduate research in H.A. Barker's laboratory at the University of California, Berkeley. The research was an extension of earlier studies in Barker's laboratory designed to establish the mechanism involved in the conversion of ethanol and acetic or propionic acids to mixtures of short chain (C_4 to C_8) fatty acids by *Clostridium kluyveri*, an organism that Barker had isolated from mud taken from a canal in Delft, Holland. Because ^{14}C-labeled ethanol and acetate were available, the research plan was to incubate the radioactive substrates with freshly prepared cell suspensions of *C. kluyveri*, isolate the labeled fatty acids formed, and develop procedures for their chemical degradation to determine the positions of the labeled carbon atoms. Results of preliminary studies established that ethanol was oxidized to an acetate derivative that condensed with added acetate or propionate to form intermediates that were reduced to butyrate and caproate or valerate and heptanoate, respectively [1].

During the early phase of this research, my wife, Thressa (known as Terry), and I made a trip to visit her mother and brother, who still lived on the farm near Oswego, New York, where Terry was born. After spending a few days with her family, Terry suggested that we go to her alma mater, Cornell University, for a brief visit with Dr. I.C. Gunsalus, who had served as her advisor in the Department of Bacteriology during her undergraduate studies leading to a B.S. degree. During our visit with Dr. Gunsalus (better known as Gunny), I mentioned that, as part of my graduate work, I had reviewed one of his papers in which he described the purification of tyrosine decarboxylase from bacterial extracts and showed that the activity of the enzyme was dependent on pyridoxal phosphate. When I asked Gunny how he prepared the bacterial extracts, he took me into his laboratory and showed me a large vacuum desiccator in which there was a large evaporating dish containing a batch of bacteria that had been collected from cell cultures by centrifugation. He explained that underneath the plate upon which the evaporating dish was sitting there was a substantial amount of Drierite desiccant (anhydrous $CaSO_4$). Therefore, when the desiccator was evacuated, the cell paste foamed up, froze, and with time became lyophilized. The dried cells were broken by grinding with alumina using a mortar and pestle. The broken cells were suspended in buffer and centrifuged, and soluble enzymes in the supernatant fraction were then used for his studies. I was impressed. This appeared to be a very simple procedure.

Upon my return to Berkeley, I used Gunny's procedure to make dried cell preparations of *C. kluyveri* and was delighted to find that suspensions of the dried cells were able to catalyze all of the steps involved in the conversion of ethanol and acetate to butyrate and caproate. Even more surprising was the demonstration that upon resuspension in water containing 0.03% sodium sulfide, at pH 7.0, the dried cells underwent spontaneous autolysis, and the cell-free fraction obtained by centrifugation contained all of the enzymes required for the conversion of ethanol and acetate to short chain fatty acids [2]. Needless to say, this changed my whole approach to studies on the mechanism of fatty acid synthesis by *C. kluyveri*. I became an enzymologist.

Further studies with enzyme preparations of *C. kluyveri* led to elucidation of the mechanism of short-chain fatty acid biosynthesis and oxidation and, subsequently, my studies as a postdoctoral fellow in Fritz Lipmann's laboratory (Massachusetts General Hospital, 1949) led to an understanding of the role of coenzyme A in these reactions. Terry and I joined the Laboratory of Cellular Physiology at the National Heart Institute, NIH, in 1950, where I continued studies with cell-free extracts of *C. kluyveri*. I also initiated studies in other

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doi:10.1016/j.bbrc.2003.09.178

areas of research using extracts of specialized bacteria isolated from soil enrichment cultures containing substrates of biological interest. This led eventually to my appointment as head of the Section on Enzymes in the Laboratory of Biochemistry. So my brief visit with Gunny was a turning point in my scientific career! As a result of that visit, I became an enzymologist and have had a fruitful scientific life, carrying out research designed to elucidate fundamental mechanisms involved in diverse areas of metabolism.

But, as wheels go around and around, so has my relationship with Gunny. In 1966, when Gunny was still at the University of Illinois, I spent some time there as a visiting professor and gave a series of lectures in a graduate course on the cellular regulation of metabolism. This proved to be a wonderful experience. It gave me an opportunity to spend some time with Gunny. Also, he introduced me to Gregorio Weber, who guided me in the use of fluorescence technology to study feedback inhibition of *Escherichia coli* glutamine synthetase by tryptophan and tryptophan analogs.

Gunny contributed directly to some early studies in our laboratory that opened up a new, still ongoing field of research. This research was prompted by the well-established fact that the intracellular levels of various enzymes are differentially affected by deprivation of diverse nutritional factors. In an effort to elucidate basic mechanisms involved, we compared the effects of carbon and nitrogen starvation on the levels of 22 different enzymes in *E. coli*. It was found that activities of about one-half of the enzymes decreased during starvation, whereas activities of the others either increased or remained unchanged. Significantly, the variations in enzyme levels elicited by nitrogen starvation were both quantitatively and qualitatively different from those accompanying carbon starvation. In an effort to define the mechanisms involved, we once again turned to studies with cell-free extracts and demonstrated that the starvation-induced loss of enzyme activities was associated with oxidative modifications that depended on the presence of O_2, iron (or copper), and an electron donor, NADH or NADPH, i.e. by components of a classical mixed-function oxidation (MFO) system [3]. This led to the proposition that oxidation of proteins marks them for proteolytic degradation [4], which was subsequently confirmed in studies with extracts of bacteria and rat liver [5].

This prompted further studies to identify other biologically relevant MFO systems that could promote oxidative modification of enzymes. Here again, Gunny and also Minor Coon were very helpful. Gunny provided us with homogeneous preparations of putidaredoxin, putidaredoxin reductase, and a cytochrome $P450_{cam}$, which he had isolated from *Pseudomonas putida* that had been grown on camphor. Minor provided us with purified preparations of the phenobarbital-induced cytochrome P450 (LM_2), which he had isolated from rabbit liver microsomes. In the presence of NADPH, Fe(III), and O_2, these enzyme preparations catalyzed the oxidative inactivation of numerous enzymes. I was delighted with the opportunity to summarize results of these studies [6] at a special meeting on Experiences in Biochemical Perception, honoring I.C. Gunsalus for his extraordinary contributions to diverse fields of science. I was pleased also by an invitation to summarize these results at the 4th International Conference on Cytochrome P-450, held in Kuopio, Finland (1982) [7], likely because Gunny and Minor Coon were both members of the International Scientific Advisory Board for that meeting.

In the course of our studies on protein oxidation, I became aware of the fact that aging is associated with the accumulation of inactive or less active, more heat-labile forms of many enzymes, including some of those that we found to be particularly sensitive to oxidative modification. This led to the proposition that the observed age-related changes in enzyme activities might reflect metal-catalyzed oxidation by MFO systems [8]. Consistent with this concept, we showed that the oxidation of purified enzymes from young animals led to changes in activity and heat stability comparable to the age-related changes observed for enzymes from old animals [9]. In continuing studies, it was demonstrated that among other changes, metal-catalyzed oxidation of proteins leads to conversion of some amino acid residues to carbonyl derivatives [10]. Therefore, highly sensitive chemical and immunological methods for the detection and quantitative measurement of protein carbonyl groups were developed [11]. Using these methods, it was established that protein oxidation is associated with many age-related changes in enzyme activities and that protein oxidation is also associated with the development of a number of neurological diseases (see [12] for review).

Among his many other outstanding scientific activities, Gunny served as assistant secretary general of the United Nations Industrial Development Organization from 1986 to 1989. During this period, he spent part of the time in New Delhi, India, and part of the time in Trieste, Italy. He also served as founding director of the International Center for Genetic Engineering and Biotechnology.

In 1989, Terry and I were invited to participate in an International Symposium on Biological Oxidation Systems in Bangalore and in satellite symposia held at the Center for Cellular and Molecular Biology in Hyderabad. During our trip to India, Gunny arranged for us to visit several academic institutions and sites of historical interest.

Thus, during 55 years of research in biochemistry, I have had many highly fruitful interactions with Gunny. Not only has he contributed directly to several of my investigations, but his support has contributed greatly to national and international recognition of my science.

Furthermore, Gunny has a passion for good wine and, over the years, Terry and I have enjoyed many evenings together with Gunny talking about science over a nice bottle of wine.

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